

## Haematological Parameters of Broilers Fed *Moringa oleifera* Leaf Supplemented Feed Following Challenge with a Very Virulent Infectious Bursal Disease Virus

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### ABSTRACT

A study was conducted to evaluate the haematological parameters of broilers fed *Moringa oleifera* leaf supplemented feed and had been challenged with a very virulent infectious bursal disease virus. Two hundred and forty day-old Ross 308 hybrid broiler chicks were assigned into four groups (A,B,C,D) of 60 chicks each in a deep litter type housing. While groups A and B were fed with formulated broiler starter and finisher containing 5% *Moringa oleifera* leaf, groups C and D were fed with formulated broiler starter and finisher without *Moringa oleifera* leaf. Groups A and C were vaccinated intramuscularly against infectious bursal disease (IBD) at 14 and 21 days of age, and Newcastle's disease at 18 days of age. Groups A, B and C were intraocularly challenged at 35 days of age with 0.05 ml of a live vvIBDV. Groups B and D served as positive and negative control respectively. Blood was collected from 10 broilers in each group via the wing vein at 35, 38 and 42 days of age for various haematological indices. A significant decrease in the values of lymphocytes counts in group B at 38 days of age was observed. Heterophil/lymphocyte ratio had significantly decreased ( $P<0.05$ ) in group D at 38 days of age. Packed cell volume significantly decreased ( $P<0.05$ ) at 38 days of age in groups A, B, C and D and subsequently increased ( $P<0.05$ ) by 42 days of age in groups B, C and D. Red blood cell count was significantly decreased ( $P<0.05$ ) in group B and C at 38 days of age, while haemoglobin concentration significantly increased ( $P<0.05$ ) at 42 days of age in group B and D. Feeding broilers with 5% MOL supplemented diet without vaccination did not prevent vvIBDV from causing a decrease in lymphocyte count 3 dpi in broilers of group B.

**Keywords:** *Moringa oleifera* leaf, vvIBDV, PCV, Lymphocyte, Haemoglobin

### INTRODUCTION

Haematology has been defined as the study of blood. It is an important part of clinical pathology as well as diagnostic process. Haematological analysis has been widely used as indices in assessing the health status of an animal (Graczyk et al., 2003; Uchehgbu et al., 2010) and

the resultant changes are important in assessing the response of animal to various pathological, nutritional and physiological situations (Akpodiete and Ologhobo 1998; Khan and Zafar 2005; Owen et al., 2009).

Poultry diseases such as infectious bursal diseases (Panigraphy et al., 1986; Juranova et al., 2001), avian

coccidiosis (Koinarski et al., 2001), Newcastle disease (ND) (Galindo-Muniz et al., 2001; Oladele, et al., 2012), fowl typhoid (Kokosharov and Todorova 1987; Barde et al., 2015) and Mycoplasmosis (Branton et al., 1997; Burnham et al., 2003; Ahmed et al., 2015) greatly influence haematological parameters of avian species.

Differential leucocytes are used as indicators of stress response and are sensitive biomarkers that are vital for immune functions. Bacterial and viral diseases affect the total number of white corpuscles thus; the proportion among the various types of white corpuscles and the percentages of the different types of white corpuscles in healthy birds, have therefore been modified in sick birds (Wendy and Jean-Marc 1992; Post et al., 2007; Muhammad and Oloyede, 2009; Barde, et al., 2015). Infectious bursal disease virus has been reported to cause alterations in different haematological parameters of poultry (Zeryehun, et al., 2012). In birds, clinical signs of illness are often delicate; therefore, clinical chemistry is essential to assess cellular changes (Ritchie, et al., 1994).

*Moringa oleifera* leaf (MOL) in the diets of broilers has been shown to have a significant effect on the blood parameters of broilers (Oghenebrorhie and Oghenesuvwe, 2016), and Mekanjuala et al. (2014) had earlier observed a numerical increase in the values of Packed cell volume (PCV), Red blood cell (RBC) and haemoglobin (HB) of broilers fed with MOL supplemented diets. The absences of hematological values of broilers fed *Moringa oleifera* leaf (MOL) supplemented diet and then challenged them with vvIBDV demand this study. Therefore, this study was aimed at assessing the effect of dietary MOL feed supplement on haematological parameters of broilers challenged with a very virulent infectious bursal disease virus (vvIBDV).

## **MATERIALS AND METHODS**

### **Study location**

The study was conducted at the Poultry Research Unit of the Faculty of Veterinary Medicine, Ahmadu Bello University Samaru, Zaria, Kaduna state, Nigeria.

### **Ethical approval**

Approval this research was sorted from the ethics committee of the Ahmadu Bello University, Zaria and guidelines for the care and humane handling of animals were strictly followed throughout the study (FASS, 2010).

### **Collection and processing of *Moringa oleifera* leaf**

*Moringaoleifera* leaf was harvested during the early flowering stage of the plant from orchards in Potiskum,

Yobe State. The stem together with the branches of the Moringa trees were cut down and spread out under shade for it to dry at room temperature for 5 days. The MOL were then manually separated from the stem and branches by hand and grounded into powder using a milling machine.

### **Mineral analysis**

The analysis of the mineral content of MOL was carried out based on the procedure of AOAC (1990) to determine minerals such as calcium, phosphorus, magnesium, iron, sodium, zinc, copper, selenium, potassium, and manganese components of the leaf.

### **Phytochemical analysis**

Qualitative and quantitative analysis of MOL was done, based on the method described by Sofowora (1993), to ascertain the presence of tannins, phytates, saponins, oxalates, cyanides, alkaloids, carbohydrates, flavonoids, steroids, terpenoids, phenols and phylobatanins.

### **Proximate analysis**

The method described by the AOAC (1990) for the proximate analysis of the MOL was used to verify the proportion of carbohydrates, crude protein, fats, fibre, ash, moisture and metabolizable energy.

### **Feed formulation and analysis**

Following shade drying of MOL, it was milled with a hammer mill and sieved with 3 mm mesh to obtain *Moringaoleifera* leaf meal. A 22% and 20% crude protein broiler starter and broiler finisher mash respectively were formulated [with 5% MOL (Olugbemi, et al., 2010) forming part of the feeds ingredients for group A and B] using Pearson square and milled in a toll mill in Zaria. The feed was subjected to analysis based on the method described by the AOAC (1990) in the Feed analysis laboratory of the Department of Animal Science, Ahmadu Bello University Zaria, to determine the level of metabolizable energy, crude protein, crude fibre, moisture, ash content, and dry matter.

### **Experimental chicks and housing**

A total of 240 day-old Ross 308 hybrid broiler chicks were obtained from a reputable commercial hatchery located in Yola, Nigeria. The chicks were brooded in a conventional open-sided house which was properly disinfected before the arrival of the chicks (deep litter system of management with wood shavings as litter material, feeders and drinkers were provided) with cyclic temperatures. The chicks were individually weighed with

a weighing balance and assigned in a completely randomised design into four different groups A, B, C and D of 60 chicks each (each pen has a floor space of 3×4 m). A 100-watt bulb was provided in each of the compartment to supply light and heat during brooding.

### **Experimental design**

Groups A and B were fed with broiler starter and finisher diets each containing 5% MOL, while groups C and D were fed with broiler starter and finisher feed without MOL. Groups A, B and C were challenged at 35 days of age with a vvIBDV. While group B served as the positive control, group D served as the negative control. All the groups were fed for 49 days (7 weeks) with feed and water provided *ad libitum*.

### **Vaccines and vaccination**

Inactivated vaccines against IBD (Virsin 122, oil emulsion, Biovac limited, Isreal, Batch 1- 382222) and inactivated vaccines against ND (oil emulsion Komorov strain, Biovac limited, Isreal, Batch 1- 422222) were obtained from a reputable Veterinary Pharmaceutical supplier in Jos, Nigeria. Broilers in groups A and C were vaccinated on the thigh muscles intramuscularly with 0.5 ml of killed IBD vaccine on 14 and 21 days of age respectively, while vaccination against ND was done with a killed ND vaccine (0.5 ml) on the thigh muscles intramuscularly on 18 days of age.

### **Challenge infectious bursal disease virus**

At 35 days of age, all the broilers in groups A, B and C were challenged intraocularly with 0.05 ml of a live vvIBD virus. The IBD virus used for the challenge was a field strain of vvIBDV obtained from previously vaccinated layers that died of natural outbreak of IBD. Sixty five per cent of commercial cockerels were inoculated at 30 days of age with 50 µl of bursal suspension (v/w) in phosphate buffer saline (PBS) (pH 7.4) died. One millilitre of bursal suspension (v/w) in PBS (pH 7.4) contained  $10^{9.76}$  CID<sub>50</sub> of IBDV.

### **Collection and processing of blood**

Blood samples of the broilers were collected when the chicks were 35, 38 and 42 days of age from all the groups for haematological assay. On each blood collection day, 10 broilers from each group that had been previously randomly selected and marked were bled via the brachial vein using a 25-gauge sterile needle on a plastic disposable 5 ml syringe. Two ml of blood were collected after the birds were properly restrained by an assistant and then emptied into a commercially available sample bottle

containing ethylene diamine tetra acetic acid for haematology. Prior to this, the area around the brachial vein was swabbed with 70% methanol to obtain optimal disinfection and to allow for easy access to the vein and for the collection of blood. Each of the sample bottles were properly labelled using a permanent marker. Direct contact with blood was avoided by the use of hand gloves and laboratory coat.

### **Determination of packed cell volume**

The Packed Cell Volume (PCV) was determined using a standard technique as described by Rehman *et al.* (2003). Non-heparinized capillary tubes were filled up to about ¾ of its length from one end and the second end was heat-sealed using Bunsen burner. The blood in the sealed capillary tubes was then centrifuged for 5 minutes at 4,383 x g using the Saitexiangyi TG12MX<sup>®</sup> Microhaematocrit centrifuge machine. Then the proportion of cells in the total volume of blood was measured and recorded as a percentage using the Hawksley<sup>®</sup> Micro-haematocrit reader.

### **Estimation of haemoglobin concentration**

Haemoglobin concentration was assayed colorimetrically as cyanmethemoglobin (Drabkin, 1945). Five ml of hemiglobincyanide (HICN) (Drabkin) solution were measured using a 5 ml syringe into plastic test tubes. Twenty µl of blood was measured using a micropipette and was added to the Drabkin solution in the test tube and properly mixed by gently shaking the test tube. It was centrifuged at 1,509 × g for 15 minutes to separate the empty Red Blood Cell (RBC) from interfering with the reading. The supernatant was separated into a sample bottle. The supernatant was absorbed into the haemoglobin meter (XF-C, China). After the wiggling pump stops working, the value displayed on the screen was recorded in g/dl as the haemoglobin concentration.

### **Determination of red blood cell and total white blood cell count**

RBC and Total White Blood Cell (TWBC) counts were determined with the Natt-Herrick solution (1:200 dilution) and the improved Neubauer haemocytometer (Campbell and Ellis, 2007) as both counts can be prepared directly from the same sample placed in the haemocytometer.

The heparinised blood samples were slightly agitated and the RBC diluting pipette was used to pipette the blood to the 0.5 marking. The tip of the pipette was cleaned properly using a tissue paper without touching the distal opening of the pipette tip with tissue, as this will cause a

capillary shift of blood into the tissue. The diluting solution (Natt-Herrick) was also pipette to the 101 marking (1:200) without entirely immersing the pipette tip into the diluting fluid. The mixture was well shaken for 1 minute to obtain equal distribution then emptied into a clean sample bottle. The Neubauer haemocytometer and cover slip were cleaned using a dry, lint free cloth. The cover slip was properly placed on the haemocytometer.

The mixture of Natt-Herrick solution and the blood sample was then agitated a little and a capillary tube was used to withdraw a small aliquot. Both sides of the haemocytometer were filled up (charged) by gently touching the intersection between the cover slip and haemocytometer with the loaded capillary tube avoiding air bubbles and under-filling or over-filling, and then left for 5 minutes for the cells to settle down. The light microscope (Olympus-XSZ-107BN), at low power magnification ( $\times 40$ ) was used to view the cells and counting was done using the tally counter.

For TWBC count, the WBC in the four outer large squares of the haemocytometer were counted and calculated using the formula:

$$N/20 = WBC \times 10^9 / l$$

Where N = Number of WBC counted in the four outer large squares (or in 64 small squares).

For RBC count, the cells contained in the four corners and central squares in the mid-section of the haemocytometer were counted. Following the "L" rule: cells that touch the centre triple lines of the ruling on the left and the bottom sides were counted but the cells that touch the centre triple lines of the ruling on the right and the top sides were not counted. The RBC count was calculated using the formula:

$$N/100 = RBC \times 10^{12} / l$$

Where N = Number of RBC was counted in the five squares in the mid-section of the haemocytometer (or in 160 squares). Note that both charged sides of the haemocytometer were counted for both the RBC and TWBC and the average calculated.

### **Preparation of smears for differential leucocyte count and thrombocytes estimation**

From the blood sample collected in all the birds, a pair of smears for each blood sample was made. A small drop of blood was immediately used for the preparation of blood smears each using the standard slide-to-slide technique. The air-dried smears were properly labelled using a pencil on the frosted end of the slide and then fixed in a fixing jar containing 70% methanol for 3 minutes and air-dried.

Staining was done by flooding the smears with Wright-Giemsa stain for 3 minutes. An equal amount of Sorensen's buffer (pH of 6.8) was added then mixed gently by blowing using a pipette until green metallic sheen forms on the surface. The smear was allowed to stand for further 6 minutes. The smears were rinsed with the Sorensen's buffer and allowed to stand for a minute for differentiation. The stained slides were then washed copiously with the Sorensen's buffer and the back of the smears were wiped with tissue paper to remove the excess stain and allowed to air dry. The slides were then neatly packed into a slide box until viewing.

Examination of the stained blood smears for differential leucocytes count was done using a light microscope (Olympus-XSZ-107BN) under high-power magnification with oil immersion ( $\times 1,000$ ). One hundred WBC were counted and classified based on their morphologic features (Campbell 1988; Hawkey and Dennet 1989; Campbell and Ellis, 2007). The counting was done using the Marble<sup>®</sup> Blood Cell Calculator. The differential WBC count was then expressed as a percentage of the individual cell group. The percentage of each cell was then converted into absolute numbers by reference to the total WBC using the formula:

$$\frac{\text{Percentage of WBC counted} \times \text{TWBC}}{100} = \text{Absolute Number} \times 10^9 / l$$

An estimated thrombocyte count was obtained from the stained blood film using the same formula for the indirect estimation of total WBC (Campbell, and Ellis, 2007). Valid and reliable results were not obtained where there was evidence of thrombocytes clumping. The absolute number of thrombocytes was estimated by using the formula:

$$\frac{\text{Number of thrombocytes counted} \times \text{TWBC}}{100} = \text{Absolute Thrombocytes} \times 10^9 / l$$

### **Data analyses**

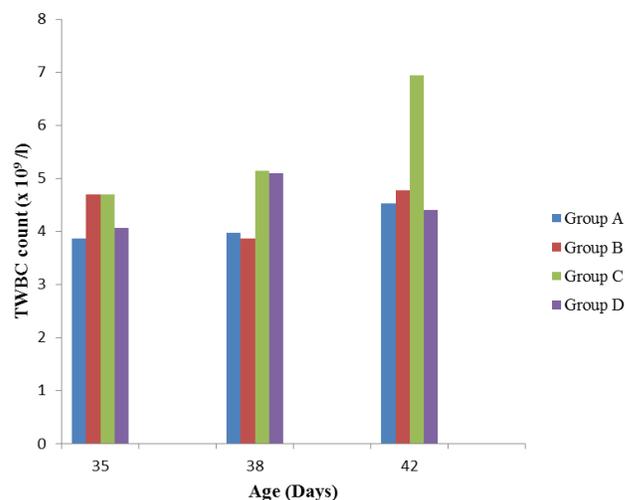
The results of the haematological values were expressed as means  $\pm$  standard deviation. They were further subjected to repeated measure one way analysis of variance (ANOVA) followed by post-hocDunnnett's control test for multiple comparison. Analyses are considered significant at  $p < 0.05$  using Statistical Package for Social Science (IBM SPSS version 20) for windows.

## **RESULTS**

Haematology results had shown significant decrease in the values of PCV in group A at 38 ( $P < 0.05$ ) and 42 ( $P < 0.05$ ) days of age. The values of PCV were also

significantly decreased in group B ( $P<0.05$ ), C ( $P<0.05$ ) and D ( $P<0.05$ ) at 38 days of age, but had significantly increased ( $P<0.05$ ) by 42 days of age in group B (Table 1). Haemoglobin concentration significantly increased in group B ( $P<0.05$ ) and group C ( $P<0.05$ ) at 38 days of age and decreases significantly ( $P<0.05$ ) at 38 days of age in group D (Table 2). The value of RBC significantly ( $P<0.05$ ) decreased at 38 days of age and increased significantly ( $P<0.05$ ) at 42 days of age in group B (Table 3), while TWBC count was observed to significantly increase ( $P<0.05$ ) between groups A and C, and B and C at 42 days of age (Figure 1).

The result of this study also showed a significant increase ( $P<0.05$ ) in the values of eosinophils count among broilers in group B at 42 days of age, but no significant increase ( $P>0.05$ ) was however observed among broilers in groups A, C and D at 35, 38 and 42 days of age (Table 4). A significant decrease ( $P<0.05$ ) was observed in lymphocyte count at 38 days of age in group B (Table 5). The values of heterophil/lymphocyte (H/L) ratio had significantly ( $P<0.05$ ) decrease at 38 days of age and a significant ( $P<0.05$ ) increase was observed between 35 and 42 days of age in group D (Table 6).



**Figure 1.** Mean ( $\pm$  SD) total white blood cell count of broilers fed 5% *Moringa oleifera* leaf feed supplement (A and B), vaccinated (A and C) with killed infectious bursal disease vaccine at 14 and 21 days old and challenged (A, B and C) at 35 days old with very virulent infectious bursal disease virus

**Table 1.** Packed cell volume of broilers fed 5% *Moringaoleifera* leaf supplemented feed

Groups	A	B	C	D
	n = 10	n = 10	n = 10	n = 10
Age in days	Mean ( $\pm$ SD) packed cell volume (%)			
35	28.60 $\pm$ 1.77 <sup>a</sup>	26.30 $\pm$ 1.94 <sup>a</sup>	25.22 $\pm$ 2.28 <sup>a</sup>	25.50 $\pm$ 3.54 <sup>a</sup>
38	21.80 $\pm$ 3.46 <sup>b</sup>	21.10 $\pm$ 1.91 <sup>b</sup>	21.44 $\pm$ 1.33 <sup>b</sup>	23.30 $\pm$ 3.06 <sup>a</sup>
42	19.90 $\pm$ 2.73 <sup>b</sup>	23.80 $\pm$ 3.01 <sup>c</sup>	22.67 $\pm$ 1.87 <sup>b</sup>	26.80 $\pm$ 2.04 <sup>c</sup>
F statistics	25.528	12.585	8.952	3.028
P value	0.0000	0.001	0.003	0.079

n = total number of birds sampled, Mean ( $\pm$  SD) = standard deviation of the mean; Means having different superscripts alphabet on the same column differ significantly  $P<0.05$

**Table 2.** Haemoglobin concentration of broilers fed 5% *Moringaoleifera* leaf supplemented feed

Groups	A	B	C	D
	n = 10	n = 10	n = 10	n = 10
Age in days	Mean ( $\pm$ SD) haemoglobin concentration (g/dl)			
35	10.55 $\pm$ 2.21	10.01 $\pm$ 1.77 <sup>ac</sup>	9.81 $\pm$ 0.97 <sup>a</sup>	13.38 $\pm$ 1.47 <sup>a</sup>
38	10.78 $\pm$ 1.95	10.32 $\pm$ 0.93 <sup>b</sup>	11.39 $\pm$ 1.17 <sup>b</sup>	11.14 $\pm$ 1.47 <sup>a</sup>
42	10.77 $\pm$ 1.50	12.06 $\pm$ 2.04 <sup>ac</sup>	11.77 $\pm$ 2.00 <sup>b</sup>	13.38 $\pm$ 1.47 <sup>c</sup>
F statistics	0.053	5.499	4.280	8.924
P value	0.940	0.015	0.040	0.015

n = total number of birds sampled, Mean ( $\pm$  SD) = standard deviation of the mean; Means having different superscripts alphabets on the same column differ significantly  $P<0.05$

**Table 3.** Red blood cell count of broilers fed 5% *Moringaoleifera* leaf supplemented feed

Groups Age in days	A n = 10	B n = 10	C n = 10	D n = 10
	Mean ( $\pm$ SD) red blood cell count ( $\times 10^{12}/L$ )			
35	2.44 $\pm$ 0.44	2.49 $\pm$ 0.34 <sup>ac</sup>	2.25 $\pm$ 0.26	2.44 $\pm$ 0.63
38	2.01 $\pm$ 0.42	1.78 $\pm$ 0.35 <sup>b</sup>	1.79 $\pm$ 0.52	2.19 $\pm$ 0.82
42	2.04 $\pm$ 0.49	2.19 $\pm$ 0.51 <sup>ac</sup>	2.09 $\pm$ 0.29	2.10 $\pm$ 0.51
F statistics	2.210	8.011	3.399	0.542
P value	0.140	0.006	0.077	0.572

n = total number of birds sampled, Mean ( $\pm$  SD) = standard deviation of the mean; Means having different superscripts alphabets on the same column differ significantly P<0.05

**Table 4.** Eosinophil count of broilers fed 5% *Moringaoleifera* leaf supplemented feed

Groups Age in days	A n = 10	B n = 10	C n = 10	D n = 10
	Mean ( $\pm$ SD) eosinophil count $\times 10^9 /L$			
35	0.05 $\pm$ 0.06	0.01 $\pm$ 0.02 <sup>a</sup>	0.15 $\pm$ 0.17	0.16 $\pm$ 0.21
38	0.10 $\pm$ 0.12	0.03 $\pm$ 0.03 <sup>a</sup>	0.19 $\pm$ 0.24	0.12 $\pm$ 0.12
42	0.12 $\pm$ 0.12	0.05 $\pm$ 0.05 <sup>b</sup>	0.13 $\pm$ 0.11	0.09 $\pm$ 0.12
F statistic	4.205	7.25	0.389	0.570
P value	6.07	0.025	0.618	0.556

n = total number of birds sampled, Mean ( $\pm$  SD) = standard deviation of the mean; Means having different superscripts alphabets on the same column differ significantly P<0.05

**Table 5.** Lymphocyte count of broilers fed 5% *Moringaoleifera* leaf supplemented feed

Groups Age in days	A n = 10	B n = 10	C n = 10	D n = 10
	Mean ( $\pm$ SD) lymphocyte count ( $\times 10^9 /L$ )			
35	3.2 $\pm$ 0.26	3.87 $\pm$ 1.52 <sup>a</sup>	3.5 $\pm$ 0.34	2.2 $\pm$ 0.44
38	3.1 $\pm$ 0.32	2.67 $\pm$ 1.26 <sup>b</sup>	4.1 $\pm$ 0.40	4.0 $\pm$ 0.60
42	3.6 $\pm$ 0.62	3.77 $\pm$ 0.82 <sup>a</sup>	4.7 $\pm$ 0.70	3.3 $\pm$ 0.51
F statistic	0.335	1.338	1.165	2.160
P value	0.653	0.023	0.328	0.153

n = total number of birds sampled, Mean ( $\pm$  SD) = standard deviation of the mean; Means having different superscripts alphabets on the same column differ significantly P<0.05

**Table 6.** Heterophil/lymphocyte ratio of broilers fed 5% *Moringaoleifera* leaf supplemented feed

Groups Age in days	A n = 10	B n = 10	C n = 10	D n = 10
	Mean ( $\pm$ SD) heterophil/lymphocyte ratio			
35	0.15 $\pm$ 0.05	0.13 $\pm$ 0.06	0.19 $\pm$ 0.07	0.68 $\pm$ 0.42 <sup>a</sup>
38	0.22 $\pm$ 0.20	0.18 $\pm$ 0.10	0.14 $\pm$ 0.06	0.17 $\pm$ 0.07 <sup>b</sup>
42	0.22 $\pm$ 0.10	0.19 $\pm$ 0.09	0.20 $\pm$ 0.12	0.22 $\pm$ 0.11 <sup>b</sup>
F statistic	0.877	1.511	2.442	11.568
P value	0.387	0.251	0.118	0.007

n = total number of birds sampled, Mean ( $\pm$  SD) = standard deviation of the mean; Means having different superscripts alphabets on the same column differ significantly P<0.05

## DISCUSSION

The significant decrease in PCV and RBC observed in groups A, B and C 3 dpi in the current study could be due to anaemia resulting from haemorrhage that is usually associated with IBD infection. The findings in the present study are in accord with those of Skeeles et al. (1980) and Moss (1999). Additionally, this finding also agrees with the works of Panigraphyet al. (1986) and Kassim, (2014) who in their separate studies reported a significant reduction in the values of PCV and RBC 5 dpi with vvIBDV, when a 4 weeks old broilers and cockerels were challenged respectively. But it is however, contrary to the findings of Oladele et al. (2005) who reported an increase in PCV of broilers challenged with vvIBDV at 32 days of age. The significant increase in PCV at 7 dpi observed in group B could suggest that minerals such as iron that were found in high quantity in the MOL used for supplementing the feed fed to broilers in group B may have possibly helped in increasing their PCV. Significant increase in the values of PCV was also reported in broilers fed MOL supplemented diet (though not challenged with vvIBDV) (Tijjani et al., 2015; Emmanuel, 2016; Oghenebrorhie and Oghenesuvwe, 2016).

The increase in the concentration of haemoglobin observed in groups B and C could indicate polycythaemia that could be due to dehydration, which is a characteristic finding in IBD (Gross, 1989). It could also imply that the 5% MOL supplemented diet and the feeds without MOL were rich in iron which is responsible for the synthesis of haemoglobin. Hassan et al. (2016) also reported an increase in the values of Hb concentration when MOL was supplemented in the diet of broiler. The increase in TWBC count observed in group C could be a response to subclinical bacterial infections such as *E. coli*. Infectious bursal disease has been reported to increase the susceptibility to various bacterial infections (Niki, 1996; Shane, 1997). The significant increase observed in the values of eosinophils in group B was probably as a result of an increase from an initial absence of eosinophils at 35 days of age (pre infection with vvIBDV).

The significant decrease observed in the values of lymphocyte in group B, showed a marked lymphopenia, 3 dpi with vvIBDV. It is well known that viral infections in birds are associated with lymphopenia (Jain, 1986). This is because IBDV causes the destruction of B-lymphocytes within the bursa of Fabricius before their migration into the blood stream, thus causing the reduction in the number of lymphocytes in the blood (Weiss and Kaufer-Weiss, 1994). After infection with IBDV, an increase in the percentage of lymphocyte without lysosomes and a

decrease in lymphocytes with large single and large multiple lysosomes have been reported 3 dpi (Klucinski, et al., 1984). The finding of this work agrees with that of Oladele et al. (2005) and Kassim, (2014) who separately reported lymphopenia 6, 12, and 48 hours, and a subsequent, increase in lymphocyte counts between 120 and 144 hours post infection with vvIBDV in 4 weeks old broilers and cockerels respectively. The result of this study implies that despite feeding broilers with 5% MOL supplemented feed; the vvIBDV was able to cause destruction of B-lymphocytes.

Increase H/L ratio has been used as an important indicator of stress in birds (Gross and Siegel, 1983). Stress in birds which may vary from food or water deprivation, temperature extremes, constant light or diseases usually elevates the number of heterophils and depresses the number of lymphocytes (Gross, 1989; Mcfarlanje, and Curtis, 1989). The highly significant increase in H/L ratio observed in broilers of group D may not necessarily be due to the above mentioned stressors, because broilers used for this study were not deliberately disturbed in anyway. However, it was observed that broilers in group D had more number of males than females, though not deliberately apportioned. This high number of males could probably be the reason for the higher H/L ratio observed. This finding is in agreement with the findings of Al-Murrani et al. (1997) where he reported that male (cock) broilers had a higher H/L ratio when compared to female (Hen) broilers and suggested that, the additional stress of higher body weight in males must have attributed to the increase in the H/L ratio.

## CONCLUSION

It was concluded that, 5% MOL supplemented diet without vaccination did not prevent vvIBDV from causing a decrease in lymphocyte count 3 dpi in broilers of group B. Supplementation of broiler feed with 5% MOL and vaccination with a killed IBD vaccine did not prevent a decrease in PCV and RBC, but cause an increase in Hb concentration 3dpi with vvIBDV in groups A and B, respectively. 5% MOL supplemented diet with or without vaccination did not cause an increase in the heterophil to lymphocyte ratio in groups A and B.

## DECLARATIONS

### Author's contribution

All authors participated in making the design, performing the experiment, analyses of the data, and writing of the manuscript.

## Consent to publish

All the authors have consented to the publication of this paper.

## Competing interest

The authors declare that they have no competing interests.

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