

## **Identification of Hematological Markers Suitable for Improving Productivity of Helmeted Guinea Fowl *Numida meleagris***

**I. I. Adedibu<sup>1\*</sup>, K. L. Ayorinde<sup>2</sup> and A. A. Musa<sup>3</sup>**

<sup>1</sup>Department of Animal Science, Ahmadu Bello University, Zaria, Kaduna state, Nigeria.

<sup>2</sup>Department of Animal Production, University of Ilorin, Kwara State, Nigeria.

<sup>3</sup>Department of Animal Production, Kogi State University, Anyigba, Kogi State, Nigeria.

### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author KLA designed the study. Author IIA managed analyses of the study, wrote the protocol and the first draft of the manuscript. Author AAM performed the statistical analysis and managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

The research was designed to estimate variations in hematological variables between sex and varieties of extensively reared helmeted guinea fowl with the objective of recommending parameters that are suitable as markers during selection. The study was carried out in the Teaching and Research farm of Department of Animal Science, Ahmadu Bello University, Zaria between May and July 2012. Five varieties of helmeted guinea fowl (45 males and 45 females) that were clinically healthy were sampled for white blood cells (WBC), packed cell volume (PCV), red blood cells (RBC), hemoglobin, platelets, Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Volume (MCV). The results showed that there were no significant ( $P > .05$ ) effect of sex on WBC, MCHC, RBC, hemoglobin, PCV and MCH, but it influenced MCV ( $P = .05$ ) and platelets ( $P = .05$ ). On the other hand, there were significant ( $P = .05$ ) effects of varieties on all the hematological parameters determined in this study. Therefore, the study of variations in hematological parameters, as well as their association with economic traits, could be used to develop marker assisted technology which can be incorporated into the traditional animal breeding methodology to fast track improvement to aid selection, improve efficiency and speed of selection in breeding programs.

\*Corresponding author: Email: [iiipinyomi@yahoo.com](mailto:iiipinyomi@yahoo.com);

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## **1. INTRODUCTION**

In most African countries especially Nigeria, guinea fowl production is in its infancy [1,2] yet guinea fowl is a promising genetic resource for evolving a low input – grain saving poultry alternative for production in the developing world. The rearing of guinea fowl is thus a potential alternative poultry system. Guinea fowl is gaining importance in the Nigerian poultry industry [3] hence the need for extensive and intensive studies into every aspect that could contribute to the future improvement of this species.

Determination of hematological parameters is essential in the diagnosis of various pathological and metabolic disorders [4]. In addition, it provides highly valuable information on the physiological state of the animal [5,6]. However, hematological parameters could provide information not only for diagnostic and management purposes, but could also be incorporated into breeding programs for genetic improvement [4].

There is a lack of exact data on the hematological profiles of helmeted guinea fowl reared in Nigeria. Values for the hematology of helmeted guinea fowl have been reported [7-9] in the southern parts of Nigeria as well as it affects sex and system of production [10]. The objective of this study is to identify hematological parameters during the breeding season that are suitable as markers for both sexes and varieties of extensively-reared helmeted guinea fowl in Northern Nigeria.

## **2. MATERIALS AND METHODS**

### **2.1 Study Location**

The research was conducted on the Teaching and Research farm of Department of Animal Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

### **2.2 Sample Size and Sample Procedure**

A total of 90 birds comprising of 18 randomly selected birds (9 males and 9 females) from each of the five varieties of helmeted guinea fowl classified in an earlier study [11] comprising of Ash, Black, Pearl Ash, Pearl Black and White varieties were used for this study. The birds which had been extensively reared were sourced from seven different locations in Katsina and Kaduna States, Northern Nigeria [12]. The ranges for body weight (kg) of helmeted guinea fowl were 1.30 to 1.80 and 1.30 to 1.70 in males and females, respectively. Body weight across varieties also ranged from 1.30 to 1.80 and 1.30 to 1.70 in males and females, respectively. Blood collection was performed as described in an earlier study [13]. About 5ml of whole blood was collected by superficial venipuncture of a wing vein using a 5ml syringe. The whole blood was collected into Ethylene Di – amine Tetra Acetic acid (EDTA) bottles and transported to the laboratory for analysis within 3hours of collection.

The following variables were analyzed for by an automated system using the COULTER™LH 750 Hematology analyzer by BECKMAN COULTER, Inc, Orange county, California, United States of America;

- i) Red blood cell (RBC)
- ii) White blood cell (WBC)
- iii) Packed Cell Volume (PCV)
- iv) Hemoglobin
- v) Mean Corpuscular Hemoglobin Concentration (MCHC)
- vi) Mean Corpuscular Hemoglobin (MCH)
- vii) Mean Corpuscular Volume (MCV)
- viii) Platelet

### 2.3 Statistical Analysis

The data was analysed using General Linear Model (GLM) procedure of Statistical Analysis System [14]. The differences between sex and between varieties of helmeted guinea fowl in haematological parameters were determined using the Tukey's studentized range (HSD) test.

The statistical model used was;

$$Y_{ijk} = \mu + V_i + S_j + e_{ijk}$$

Where,

$Y_{ijk}$  = observation on the  $j^{\text{th}}$  sex of the  $i^{\text{th}}$  variety of helmeted guinea fowl

$\mu$  = overall mean.

$V_i$  = the effect of  $i^{\text{th}}$  variety of helmeted guinea fowl ( $i=1-5$ )

$S_j$  = the effect of  $j^{\text{th}}$  sex of helmeted guinea fowl ( $j=1-2$ )

$e_{ijk}$  = residual error

## 3. RESULTS AND DISCUSSION

### 3.1 Sex Differences in Hematology of Helmeted Guinea Fowl

The haematological parameters determined in the male and female guinea fowls are indicated in Fig. 1. The WBC and MCHC were slightly higher in females while RBC, hemoglobin, PCV and MCH in males, though the differences were not significant ( $P>.05$ ). However, the males had significantly ( $P=.05$ ) higher MCV than the females while the females had significantly ( $P=.05$ ) higher platelet value.

The values of PCV in this study were similar to values obtained for the Pearl helmeted guinea fowl [10,15,16]. The RBC values of both sexes were similar to those reported [15] and within the general reference range of avian ( $2.1-5.5 \times 10^{12}/l$ ) [17]. Hence, all these values were representative of a healthy helmeted guinea fowl. Hemoglobin is a component of the red blood cells and in this study it was also within the reference range for avian (12.20–20.00g/dL) [17]. This result was also consistent with the report of no sex effect on WBC, RBC, MCHC, PCV, MCV, MCH and hemoglobin in yellow – legged gulls [18]. Similarly, no significant ( $P>.05$ ) influence of sex on RBC, hemoglobin and WBC was reported in male and female Ring – necked pheasants [19] and in turkey [20]. This result however differed from those obtained by some authors who reported significant effect of sex on blood hematology in helmeted guinea fowl [10] and in Sudanese indigenous chicken [21]. In their report, PCV, RBC and hemoglobin were higher ( $P=.05$ ) in males than females and these differences were

attributed to increased courting of the females by the males during their breeding season which was when they were bled. Hemoglobin and PCV are influenced by reproductive hormones: androgen and estrogen depress erythropoiesis [22] whereas androgen and thyroxin stimulates erythropoiesis [23].

RBC, PCV and hemoglobin are responsible for enhancing oxygen delivery to the testes of naked neck cocks [21] with an improving of semen quality [24]. More so, an increased number of RBC and PCV in cocks correspond with the time of androgen production [25,26]. Thus, increase in PCV could be used to predict spermatozoa maturation in testes, sexual maturity and the start of semen production [27] while hemoglobin can be used as indicator of feed conversion efficiency [28].

WBC cleans up the debris and protects the body against invading microorganisms and body cells with mutated DNA [29]. The results were similar in both sexes and stayed within the reference range of similar birds reared extensively [19,21]. It indicated that, genetically, both sexes had the equal immune potential against diseases.

MCHC and MCH values were within the range obtained for Nigerian Indigenous chicken [30] and Sudanese Indigenous chicken [21] respectively. MCHC and MCH were not affected by sex which indicated that there was similar cellular haemoglobin content in blood samples obtained. An increase in MCHC value does not occur since RBCs do not become supersaturated with hemoglobin. On the other hand, a reduced value of MCHC usually indicates a deficiency anemia from an insufficiency of hemopoietic factors [17]. MCH has been reported as a not such useful index as the MCHC [17].

In this study, sex significantly influenced MCV and platelets. MCV values in both sexes were lower than those reported for the Sudanese indigenous chicken and helmeted guinea fowl [10,21] but close to the value reported for helmeted guinea fowl ( $176.73 \pm 33.08$ fL) [15]. MCV gives an indication about the type of anemia and it may also suggest the etiology of the disease process [17]. An increase in this value is useful for indicating a regenerative anemia. In a non-regenerative anemia, the cells are either normal or reduced in size.

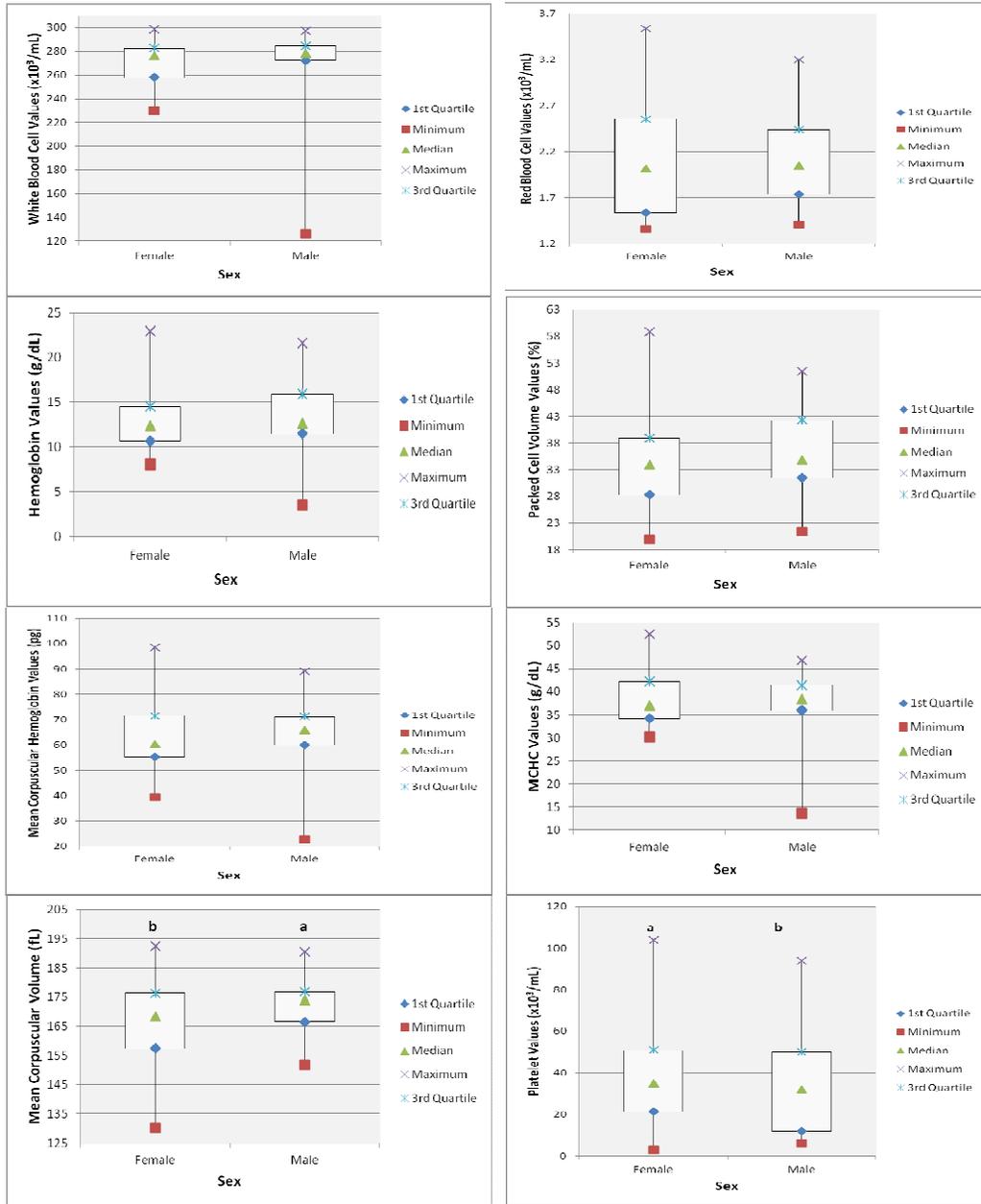
In this study, sex influenced also platelets values in this study in accordance with the reports on avian hematology [23,31,32]. Platelets are essential for the repair of blood vessels when damaged; they also provide growth factors for healing and repair [29]. Low platelet concentration suggests that the process of clot-formation (blood clotting) will be prolonged resulting in excessive loss of blood in case of injury [33]. The results indicated that both sexes have a different ability to prevent hemorrhages. Hematological parameters that have bearing on productivity of livestock should be incorporated in the formulation of selection indices for optimum productivity [34].

### **3.2 Differences in Hematology of Helmeted Guinea Fowl Varieties**

The values of the hematological parameters in five varieties of helmeted guinea fowl are indicated in Fig. 2. There were significant ( $P=.05$ ) differences between the five varieties of the helmeted guinea fowl in all the hematological parameters determined in this study.

The Black, Pearl Black, Pearl Ash and White varieties were similar in WBC, RBC, hemoglobin and PCV but they had significantly ( $P=.05$ ) higher values for the WBC than Ash variety. The MCV values were similar ( $P>.05$ ) for Ash, Black, Pearl black and White but the Pearl Ash had significantly ( $P=.05$ ) higher values than the other four varieties. The MCH

value of the Pearl Ash was significantly ( $P=.05$ ) higher than the other four varieties. The values in the Black, Pearl Black and White were similar ( $P>.05$ ) but higher ( $P=.05$ ) than in the Ash variety. The platelets value was significantly ( $P=.05$ ) higher in the Black than in the other four varieties. The White variety also had a significantly ( $P=.05$ ) higher value while, all the other ones had similar values ( $P>.05$ ).



**Fig. 1. Influence of sex on hematological parameters in helmeted guinea fowl**  
<sup>ab</sup>columns with different superscripts differ significantly ( $P=.05$ )

The test for WBC levels is very important to detect and measure the ability of the body to destroy diseases. Significant differences were observed in this study between varieties of the helmeted guinea fowl. It indicated that, although the animals appeared clinically healthy, some undiagnosed infections could be still present. The five varieties of helmeted guinea fowl had varying abilities to fight diseases, as indicated by the varying levels of white blood cells. A high level of white blood cells could also be a factor that enhances the resistance of the helmeted guinea fowl compared to other poultry. WBC was similar to the range reported for Nigerian local and exotic chickens ( $21.50\text{--}28.23 \times 10^3/\mu\text{L}$ ), [35] and the birds may be immunologically challenged which may be largely informed by the associated inherent resistance to tropical diseases [35]. It is also noteworthy that the subsisting ecological condition support several vectors and parasites of economic significance.

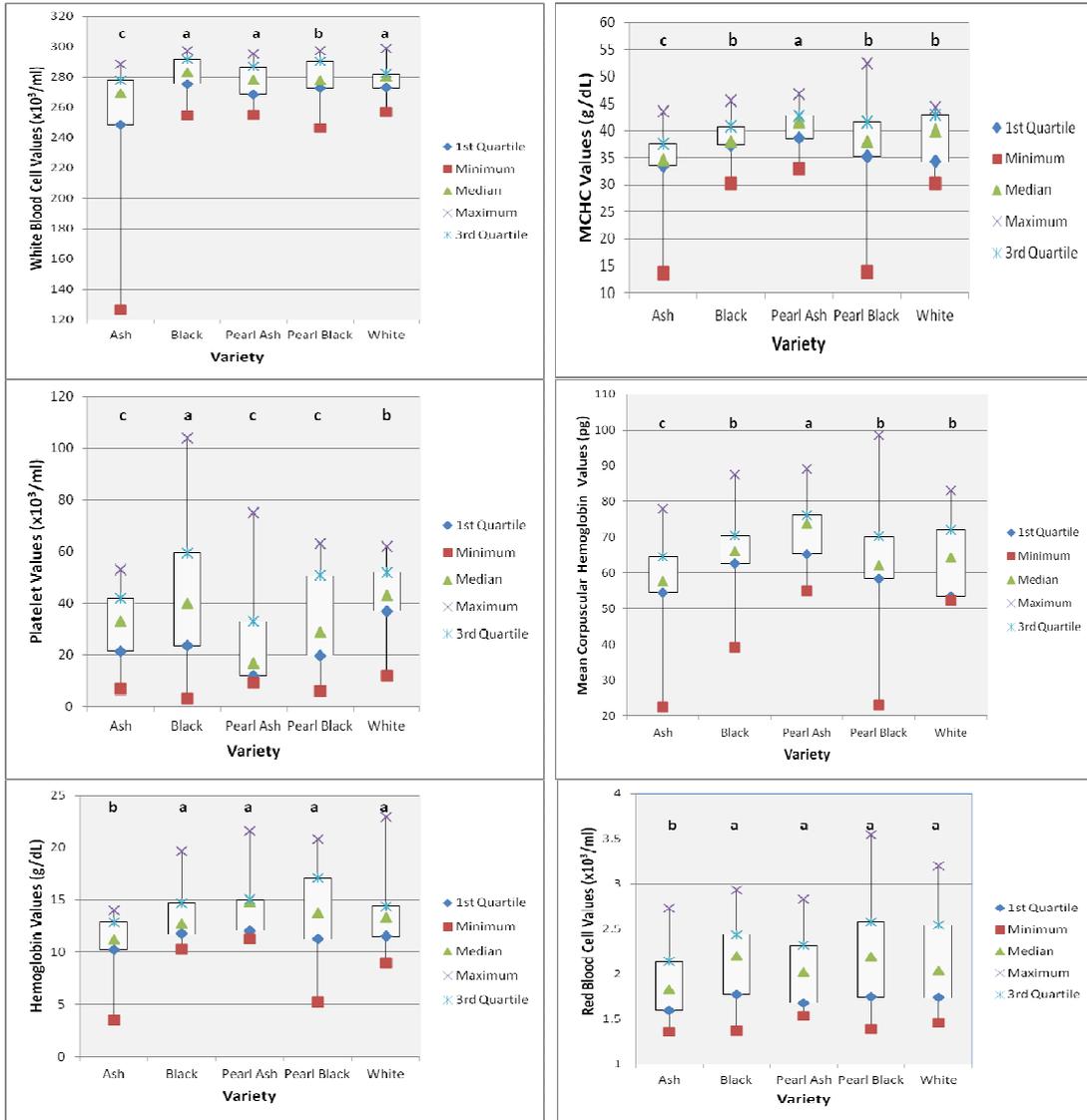
The RBC is a function of other components such as PCV, hemoglobin, MCH and MCHC. In this study, the variety of the helmeted guinea fowl influenced the values. The Black, Pearl Ash, Pearl Black and White varieties have superior values of red blood cells to the Ash variety. This indicated varietal differences since all the birds used were of similar ages, all sexually matured and the females were in – lay and under same environmental factors. The RBC values in this study were within the range of  $2.20 \times 10^{12}/\text{L}$  [36] and  $2.15 \text{ (T/L)}$  [15] for helmeted guinea fowl and  $2.10\text{--}2.58 \text{ (T/L)}$  for game birds. They were however higher than  $1.13 \times 10^{12}/\text{L}$ , data which is found in another report for helmeted guinea fowl [10]. The ideal range of red blood cells of helmeted guinea fowl during the reproductive phase based on this study was  $2.06\text{--}2.20 \times 10^6/\mu\text{L}$  since there were no significant differences between varieties except in the Ash.

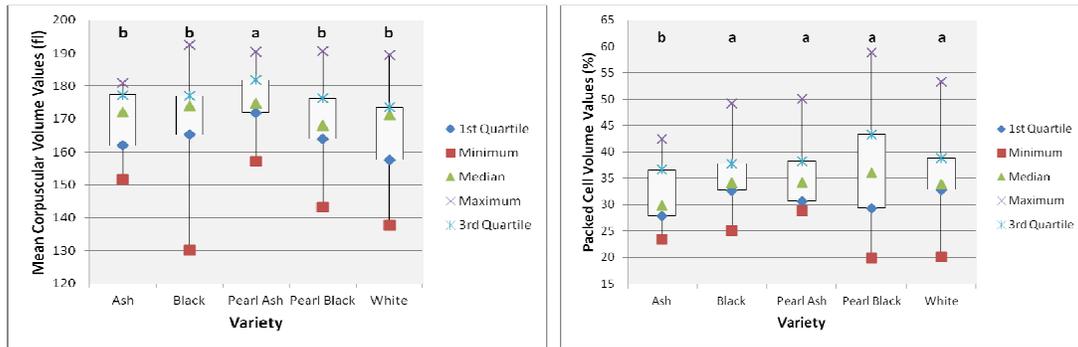
Packed Cell Volume (PCV) or haematocrit is the volume of the red cells (erythrocytes) in the blood; hence the pattern of significant differences was similar. The PCV values obtained in this study ( $35.71\text{--}37.14\%$ ) can be regarded as normal as the birds were in – lay and the values were similar to previous reports for laying Pearl helmeted guinea fowl [29] and adult helmeted guinea fowl [10,15] where values of  $34.52\%$ ,  $36\%$  and  $37\%$  respectively were reported. The values were, however, lower than the reference range for psittacines,  $40\text{--}55\%$  [32] and  $42.60 \pm 1.60\%$  for frizzled feathered Nigerian indigenous chicken [37] which have similar phenotypic features as helmeted guinea fowl. This is because higher PCV values are usually due to a higher weight gain as well as reduction in heat load [38]. Selection of helmeted guinea fowl with significantly high PCV would therefore lead to a boost in the growth and productive life [37]. The range of values ( $35.71\text{--}37.14\%$ ) obtained in this study, except in the White variety could be used to establish the ideal range for PCV as a reference range for the helmeted guinea fowl during the breeding season.

The MCV values obtained in this study were within the reference range for small psittacines,  $121\text{--}200\text{fl}$  as reported [17] and could be used as reference values for the helmeted guinea fowl during breeding. The values, obtained in this study were similar to  $176.73 \text{ (fL)}$  [15] for helmeted guinea fowl but higher than the  $130.82\text{--}141.19 \text{ (fL)}$  [37] for Nigerian local and exotic chickens. MCV values were also higher than  $86.99$ ,  $105.88$  and  $116.66$  cubic micron for Fayoumi, Assil and local chicken, respectively [31].

Hemoglobin is also a component of RBC hence, the pattern of significant differences was similar. Except for the White variety, the other four varieties had similar hemoglobin values in this study. This indicated that the birds were in a similar health status since variations in hemoglobin can be due to: living at high altitudes, excessive/under production by bone marrow of blood cells, anemia due to nutritional deficiency, destruction of blood cells internally, liver tumor (hepatomas), kidney tumor and lung diseases [39]. The range ( $13.61\text{--}$

14.19g/dL) obtained could be used as a reference for the breeding of *Numida meleagris* during the breeding season in Northern Nigeria. The reference range, as obtained in this study, was similar to Nigerian local and exotic chickens (12.36–16.37g/dL)[37]. This indicated that the physiological role of hemoglobin with oxygen and the transportation of gases (oxygen and carbon dioxide), to and from the tissues, of the body has been maintained and was normal [37].





**Fig. 2. Influence of variety of helmeted guinea fowl on hematological parameters**  
<sup>abc</sup> columns with different superscripts differ significantly ( $P=0.05$ )

The MCHC values in this study indicated significant differences between varieties. MCHC denotes the volume and character of hemoglobin and it is the most accurate between red blood cell indices. The values (33.92–40.63g/dL) in this study could serve as reference for breeding *Numida meleagris* in Northern Nigeria and recommending them as a range because Pearl Ash variety had a significantly higher value while the Ash variety had a significantly lower value. The values obtained were similar to previous reports for game birds: helmeted guinea fowl *Numida meleagris* 35.44%; common pheasant *Phasianus colchicus* 31.27%; Chukar *Alectoris chukar* 30.40%; Wild turkey *Meleagris gallopavo* 30.71% (Strakova et al., 2010). Wide variations (33.92–40.63g/dL) in the results could be due to age of the cells since the values were high. It has been reported that if cells are old and not replaced often (sperocytosis) and *In vivo* and *In vitro* hemolysis occur high MCHC values result; and if the values are obtained when there are too many young RBC (reticulocytes) or when there is iron deficiency, low values are obtained [39]. The MCHC values (33.92–40.63g/dL) in this study were within the reference range of 28–38g/dL for psittacine [17].

The MCH values in this study differed significantly between varieties of the helmeted guinea fowl. The MCH value followed a similar pattern as red blood cells; since it is dependent on the number of RBCs. The test defines the type of anemia and cause of anemia if present in species. Wide variations in MCH values could be due to anemia as a result of vitamin B<sub>12</sub> or/and folate deficiency, ineffective production in the bone marrow, recent loss and replacement by newer reticulocytic cells from bone marrow and *In vivo* or *In vitro* hemolysis [39]. Similar values of 60.88 [36] and 62.07(pg) were reported which were lower than the 120.33(pg) in another study [10]. The values (64.19–71.56pg) obtained in the present study can be recommended as a range for the extensively raised helmeted guinea fowl.

The platelets in this study were significantly ( $P=0.05$ ) different across variety. This indicated that varieties had differing abilities to prevent excessive bleeding from occurring.

#### 4. CONCLUSION

In conclusion, we found that MCV and platelets significantly differed with sex while RBC, WBC, PCV, hemoglobin, MCHC, MCH, MCV and platelets differed with varieties of extensively reared guinea fowl during breeding season in northern Nigeria. Selection of *Numida meleagris* males with high RBC, hemoglobin and PCV for breeding may improve

fertility due to improved semen quality, and androgen production. PCV values may be useful markers for predicting spermatozoa maturation in testes, sexual maturity and the onset of semen production. The WBC could serve as a marker indicating immunity and inherent resistance to tropical poultry diseases and parasites while hemoglobin and MCV values similar to or above (but within the reference range of avian) those obtained in this study could be suitable markers for evaluating and improving the selected population of helmeted guinea fowl for feed conversion efficiency and adequate oxygen supply for maintenance of body functions. PCV can serve as a marker for selection of helmeted guinea fowl for improved growth and productive life of the bird through improved weight gain and heat – tolerance.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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