

HAEMATOLOGICAL PARAMETERS DYNAMICS OF DEVELOPING *GALLUS GALLUS DOMESTICUS*

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ABSTRACT

The blood parameters of 12 broiler chickens (Gallus gallus domesticus) were determined at the ages of 1, 3, 5 and 7 weeks old, for follow-up of the developmental dynamics in their haematological profile. Blood samples were collected from chickens at 7, 21, 35 and 49 days intervals. Total number of WBC, RBC, PCV, Hb, MCV, MCH, MCHC together with absolute count of differential leucocytes (neutrophils, monocytes, eosinophils, lymphocytes and basophils) was determined. Significant age effects (p<0.05) was observed on RBC, PCV, Hb, MCV, MCH, MCHC and WBC. With increasing age, Hb, PCV, MCV, MCH and WBC increased significantly and RBC decreased significantly (p<0.05), while MCHC and differential leucocyte count had no significant variation (p>0.05). Advancement of age led to increasing body weight. There was a high correlation between body weight and some blood parameters of the broiler chicken. Weight accounted for 19.79 %, 11.87 %, 37.49 %, 36.09 %, 34.92 % and 25.77 % of the variations in PCV, Hb, RBC, WBC, MCV and MCH respectively. Age therefore has effects on the haematological parameters and also determines the body weight of broiler chicken (Gallus gallus domesticus).

Keywords: Development, Broiler chicken, Haematological parameters, Age and growth

INTRODUCTION

Haematological studies are very important in diagnosing the structural and functional status of the animal's body (Elagib *et al.*, 2011). Haematological changes are routinely used to determine various influences of environmental, nutritional and or pathological factors (Graczyk *et al.*, 2003). There is limited information concerning the normal blood profiles of different indigenous chickens of varying age and for husbandry regimens in Botswana (Mushi *et al.*, 1999). A meta-analysis of haematological parameters allowed comparison of domesticated chickens (commercial or indigenous) with the ancestral red jungle fowl, with wild birds in the order *Galliformes* and with wild birds. Major

differences were observed with haematocrit (HCT)/packed cell volume (PCV). The HCT/PCV was very similar between the ancestral species from which chickens were domesticated, the red jungle fowl (*Gallus gallus*) and wild birds in the order *Galliformes* (Scanes and Christensen, 2014). Such information apart from being useful for diagnostic and management purposes could equally be incorporated into breeding programmes for genetic improvement of indigenous chickens (Kral and Sachy, 2000). For proper management of broiler chicken, it is desirable to know the normal physiological values under normal situation. For example, high PCV (%) and high Hb (g %) are indicators of high feed conversion efficiency (Nyaulingo, 2013). The haematological parameters of healthy birds are influenced by many factors

which include feed restriction and nutrient conditions (Etim *et al.*, 2014), environmental factors (Vecerek *et al.*, 2002; Graczyk *et al.*, 2003), fasting (Lamosova *et al.*, 2004), nutritional contents (Bashar *et al.*, 2010), water and feed restriction (Iheukwumere and Herbert, 2003; Boostani *et al.*, 2010), age (Talebi *et al.*, 2005), continuous supplementations of vitamin E (Traset *et al.*, 2000), administration of drugs (Squires and Julian, 2001; Sureshet *et al.*, 2012), breed (Mushi *et al.*, 1999) and aflatoxin (Oguz *et al.*, 2000). Various studies have been done on the effect of food supplements, different diets (nutrition), management system, sex and breed on haematological parameters of broiler chickens and wild birds in the order Galliformes. The objective of this study was to investigate the haematological profile of broiler chicken (*Gallus gallus domesticus*) at different developmental stages and to evaluate the correlation between growth-related dynamics of body weight and some of the blood parameters.

MATERIALS AND METHODS

Broiler Chick: In the present study, a total of thirty (30) broiler chickens of one-day old Arbor Acre AA breed, from Agric International Technology and Trade (Nigeria) Limited (AGRITED), Ibadan, Nigeria were procured from the Ogige market, Nsukka, Enugu State, Nigeria. The birds were brooded for one week in other to acclimatize to the environmental conditions at the Department of Zoology and Environmental Biology Animal House, University of Nigeria, Nsukka. The cages were kept in one house to give it identical environment and were provided with a feeder and drinker. All animals were placed in a temperature controlled room at $30 \pm 2^\circ\text{C}$. The temperature of the room was reduced by 4°C at 4 weeks of age. After the fourth week, no heating was applied. The birds were fed super starter diet (Top Feed Nigeria Limited) for the first four weeks, and super finisher diet for beginning of the fifth week to the end of the study. Feed and water was provided *ad libitum*. All chicks were given timely and adequate vaccination against common poultry viral, bacterial and protozoal diseases (Meeusen *et al.*, 2007).

Blood: Blood samples for analysis were collected early in the morning from 12 randomly sampled birds on day 7, 21, 35 and 49 from the wing vein. One milliliter of blood was aseptically collected using sterile one milliliter needle and syringe from the wing vein of the birds. Immediately after collection, blood was transferred to the EDTA anticoagulant vials to prevent clotting (Nyaulingo, 2013). During the collection of blood, needles and syringes were changed between birds to prevent contamination and not more than 1 ml blood was taken per bird in a day.

Haematology: Red blood cell (RBC) count and white blood cell (WBC) count were done using haemocytometer, by mixing a quantity of blood sample with the diluent (Turks solution). The contents were mixed up by carefully shaking horizontally for a few minutes. $20\mu\text{l}$ of blood sample was drawn into a clean dry counting chamber of haemocytometer. Precautions were taken to ensure that the space between the ruled area and the cover glass was filled without overflowing. The filled counting chamber of haemocytometer was left for 3 minutes for the cells to settle in the chamber. Using a light microscope x40 objective, total red blood cells and white blood cells were manually counted in chambers of the improved Neubauer counting chamber. The red blood cell count was expressed as $\text{RBC} \times 10^{12}/\text{l}$.

To obtain the packed cell volume (PCV), the anticoagulated blood sample was filled into heparinized capillary tubes to the three quarter level, and then sealed at one end using a sealant. The sealed capillary tubes were centrifuged at 12000 revolutions per minute for 5 minutes. Thereafter the sedimented blood cells were read using the micro-haematocrit reader in percentages.

To count the differential white blood cells (DWBC), a smear was made using a drop of anticoagulated blood sample and slide to slide technique, fixed with methyl alcohol after which it was stained using Leishman stain, washed and dried. A drop of oil immersion was placed on the lower third of the blood film and placed on a microscope.

The cells were focused with $\times 10$ objectives and counted with $\times 40$ objectives in a zigzag manner. The record was taken using a manual differential cell counter to avoid errors. The absolute number of each white cell type was calculated thus: Percentage of white blood cell counted \times total WBC / 100 = absolute number (%).

The haemoglobin concentrations (Hb) were obtained using 4 ml of Drabkin's solution dispensed into sample tube using automatic micropipette and allowed to stand for 5 minutes to allow working solution to reach room temperature. 20 μ l of whole blood was aspirated from storage tube using micropipette into the sample tube. The side of pipette tip was carefully wiped using tissue and not allowing the tissue to touch the distal opening of the pipette tip, as this will cause capillary shift of blood into the tissue. Avoiding immersing of the pipette tip into the diluting fluid as it is a poor laboratory practice. 3.5 ml of the diluted blood was decanted into cuvette then in spectrophotometer at the absorbance reading of 540 nm but before the spectrometer was tarred using ammonia solution as blank. Reading expressed as Hb g/dl.

Mean corpuscular volume (MCV), mean corpuscle haemoglobin (MCH) and mean corpuscle haemoglobin concentration (MCHC) were obtained from the red blood cell count, packed cell volume and haemoglobin concentration through the following formula: $MCV (fl) = (PCV \times 10) / RBC$, $MCH (pg) = (Hb \times 10) / RBC$ and $MCHC (g/l) = (Hb \times 100) / PCV$.

Statistical Analysis: The data obtained were statistically analyzed using one-way analysis of variance (ANOVA), Duncan new multiple range test and Pearson correlation coefficient. Test of Significant differences between treatments means were accepted at 0.05% level of probability.

RESULTS

Variations were observed in the red blood cell parameters (PCV, Hb and RBC) between weeks 1 and 7. For the duration of development, the highest level of packed cell volume (PCV) in the birds was at week 5 ($32.75 \pm 0.91\%$) which was significantly higher ($p < 0.05$) than the week 7 ($29.92 \pm 0.87\%$), week 3 ($27.87 \pm 0.92\%$) and week 1 ($24.87 \pm 0.77\%$) values. The PCV value of the birds at week 1 was significantly less ($p < 0.05$) than all other weeks of study duration (Table 1).

The haemoglobin concentration (Hb) was highest at week 7 though not significantly different ($p > 0.05$) from week 5, but significantly different ($p < 0.05$) from week 3 and 1. The Hb of weeks 1, 3 and 5 were not significantly different ($p > 0.05$). The RBC count of the birds was significantly higher ($p < 0.05$) at week 1 than weeks 3, 5 and 7. The RBC count at week 3 was significantly higher ($p < 0.05$) than for weeks 5 and 7. The MCV concentration increased significantly ($p < 0.05$) on week 3, 5 and 7 when compared to week 1. Week 5 and 7 concentrations of MCV, though within the same range, were significantly higher ($p < 0.05$) than MCV of week 3. MCH concentration for week 5 and 7 were statistically similar but significantly higher ($p < 0.05$) than MCH of weeks 1 and 3. There were no significant variations ($p > 0.05$) in the MCHC values of the birds for the duration of the study (Table 1).

Significant developmental changes in the white blood cell parameters of the birds were only observed for WBC count. The WBC of the birds at week 1 was significantly higher ($p < 0.05$) than those for weeks 3, 5 and 7 (Table 2). The weight of broiler chicken increased progressively from week 1 to week 7. Bird weights were significantly different ($p < 0.05$) in the order: week 7 $>$ week 5 $>$ week 3 $>$ week 1 (Figure 1).

Table 1: Developmental changes in red blood cell profile of broiler chicken (*Gallus gallus domesticus*)

Duration	Red blood cell parameters		
	PCV (%)	Hb (g/dl)	RBC ($\times 10^{12}/l$)
Week 1	24.87 \pm 0.77 ^c	14.57 \pm 0.83 ^b	2.70 \pm 0.05 ^a
Week 3	27.87 \pm 0.92 ^b	13.67 \pm 0.80 ^b	2.34 \pm 0.67 ^b
Week 5	32.75 \pm 0.91 ^a	15.00 \pm 0.99 ^{ab}	2.23 \pm 0.05 ^c
Week 7	29.92 \pm 0.87 ^b	18.58 \pm 1.61 ^a	2.19 \pm 0.04 ^c
Duration	MCV (fl)	MCH (pg)	MCHC (g/l)
Week 1	92.42 \pm 3.08 ^c	54.38 \pm 3.38 ^b	60.21 \pm 4.78 ^a
Week 3	115.35 \pm 4.24 ^b	57.48 \pm 3.63 ^b	50.13 \pm 3.72 ^a
Week 5	148.88 \pm 7.10 ^a	73.30 \pm 5.32 ^a	49.08 \pm 3.12 ^a
Week 7	136.80 \pm 4.22 ^a	84.74 \pm 7.16 ^a	61.69 \pm 4.73 ^a

PCV = packed cell volume, Hb = haemoglobin concentration, RBC = red blood cell, MCV = mean corpuscular volume, MCH = mean corpuscle haemoglobin, MCHC = mean corpuscle haemoglobin concentration

Table 2: Developmental changes in white blood cell profile broiler chicken (*Gallus gallus domesticus*)

Duration	White blood cell parameters		
	WBC ($\times 10^9/l$)	Neutrophil (%)	Lymphocyte (%)
Week 1	11.04 \pm 0.34 ^a	62.93 \pm 0.83 ^a	35.07 \pm 0.98 ^a
Week 3	9.28 \pm 0.06 ^b	64.00 \pm 0.83 ^a	33.53 \pm 0.79 ^a
Week 5	8.96 \pm 0.15 ^b	63.00 \pm 0.62 ^a	34.83 \pm 0.77 ^a
Week 7	8.88 \pm 0.16 ^b	64.08 \pm 0.67 ^a	33.25 \pm 0.65 ^a
Duration	Basophil (%)	Eosinophil (%)	Monocyte (%)
Week 1	0.00 \pm 0.00	1.27 \pm 0.18 ^a	1.20 \pm 0.20 ^a
Week 3	0.00 \pm 0.00	1.33 \pm 1.13 ^a	1.27 \pm 0.21 ^a
Week 5	0.00 \pm 0.00	1.50 \pm 1.00 ^a	1.33 \pm 0.23 ^a
Week 7	0.00 \pm 0.00	1.58 \pm 0.15 ^a	1.25 \pm 0.18 ^a

White blood cell = WBC

Table 3: Pearson correlation (r) of weight with haematological parameters of broiler chicken (*Gallus gallus domesticus*)

Parameters	Pearson correlation (r)					
	PCV (%)	Hb (g/dl)	RBC ($\times 10^{12}/l$)	WBC ($\times 10^9/l$)	MCV (fl)	MCH (pg)
Weight (g)	0.445**	0.344*	-0.612**	-0.601**	0.591**	0.508**
p-value	0.001	0.011	0.000	0.000	0.000	0.000

PCV = packed cell volume, Hb = haemoglobin concentration, RBC = red blood cell, MCV = mean corpuscular volume, MCH = mean corpuscle haemoglobin, MCHC = mean corpuscle haemoglobin concentration, **correlation significant at 0.01, *correlation significant at 0.05

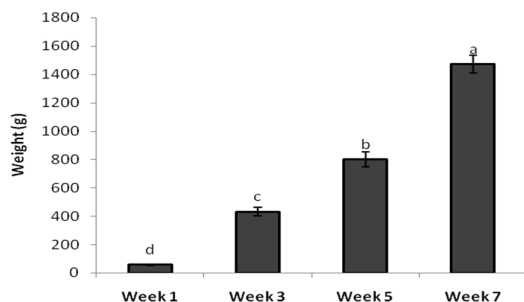


Figure 1: Developmental changes in weight of broiler chicken (*Gallus gallus domesticus*). Values with different alphabet superscript were significantly different at $p < 0.05$ from Duncan MRT

The correlation of the haematological parameters with weight of birds indicated that only six out of the twelve haematological parameters correlated significantly with weight. There were significant positive linear relationships between PCV ($r = 0.445$, $p = 0.001$), Hb ($r = 0.344$, $p = 0.011$), MCV ($r = 0.591$, $p = 0.000$) and MCH ($r = 0.508$, $p = 0.000$) and weight. RBC ($r = -0.612$, $p = 0.000$) and WBC ($r = -0.601$, $p = 0.000$) had significant negative linear relationships with weight (Table 3). Weight accounted for 19.79, 11.87, 37.49, 36.09, 34.92 and 25.77% variations in PCV, Hb, RBC, WBC, MCV and MCH respectively.

DISCUSSION

The result presented showed that as the chickens grew older, haematological values such as PCV, Hb, MVC and MCH values increased, while WBC and RBC values decreased with age. The result is consistent with the report of Tufan and Ramazan (2011) that Hb amounts and PCV values increased with the advancement of age, being lowest in the chicks and highest in the adults except for RBCs and WBCs that reduced with advancement of age.

To assess the trend of growth in chickens and other animals, body weight gain is normally used because it indicates if the chicken is growing or not. In this experiment body weight increased with age. There was a significant increase in weight difference in all the weeks in ascending order from week 1 to 7 meaning that they gained more weight as they grew. This agreed with the report of Nyaulingo (2013). The observed body weight gain of chicks may be as a result proper utilization of protein, energy, vitamin and mineral components of their diet.

This present study indicated that red blood cell counts of broiler chicken decreased as the age of the chicken increased. This maybe as a result of dietary effect, since young chicks consume less amount of feed compared to grown chickens (Coles, 1986; Sjaastad *et al.*, 2003; Talebi *et al.*, 2005; Nyaulingo, 2013). Normal red cell production requires diet containing protein, iron, copper, vitamin B₂, B₆, B₁₂ and folic acid (Nyaulingo, 2013).

The packed cell volume increased as the age of chicken increased. Although PCV is derived from red blood cell, increase or decrease in the number of RBC had no effect on the PCV. This concurred with the findings of Nyaulingo (2013) in his study on the effect of different management system on the haematological parameters of layer chickens and Talebi *et al.* (2005) on the comparative study of haematological values of broiler strains. PCV values obtained were within the physiological range of 22 – 35 % as stated by Jain (1986). PCV range of 22-28 % occurred at week 1 and 3 and 28 - 35 % at week 5 and 7 indicated that PCV values were low when the

chicks are younger and high as they advanced in age. Haemoglobin concentration increased as the broiler advanced in age. The Hb values obtained in this study were far higher than the normal physiological values of Wikivet (2012). Hb values not being in normal physiological range, indicated that there was significant effect of age on Hb concentration. Nyaulingo (2013) reported that the iron mineral contained in the diet played an important part in the synthesis of haemoglobin in the red blood cells. Hb concentration in this study increased as the RBC decreased, contrasted Nyaulingo (2013) reported increased haemoglobin concentration as chicks grew older, because of the increase in the number of RBC brought about by the increase of feed intake. The changes in haemoglobin concentration with age of chickens concurred with other reports (Islam and Rahman, 2004; Talebi *et al.*, 2005; Addass *et al.*, 2012; Nyaulingo, 2013).

Mean corpuscular volume is the expression of the average volume of individual red blood cell. Increased MCV values recorded in this study was in agreement with Talebi *et al.*(2005). The values obtained were far greater than the normal physiological ranges of Jain (1986) and Wikivet (2012). This may be as a result of reduced values of Red Blood Cells. The values obtained ranged from 92.42 to 136.80 fl and were lower than Awotwi (1990) reported MCV value of 151.26 cell/mm³ for adult Ghanaian domestic chicken and 156.8 cell/mm³ reported by Oyewale (1987) for Nigerian domestic chicken. This may be attributed to the differences in genotypes and possibly the environmental conditions under which the birds were reared.

Mean corpuscular haemoglobin is the mean mass of haemoglobin per red blood cell in a given sample of blood, so it is dependent on haemoglobin. MCH values in this study increased with advancement of age and body weight. At older age the MCH was observed to be higher than the value at younger age. Earlier studies reported inverse relationship between MCH and age; that MCH decreased with advancement in the age of birds (Islam and Rahman, 2004; Mmereole, 2004; 2009; Nyaulingo, 2013). The direct relationship

between MCH and age reported in this study is in agreement with the report of Talebi *et al.* (2005) for Ross, Cobb, Arbor-acres and Arian broiler strains. The MCH values obtained in this study is far greater than the normal physiological ranges of Jain (1986). This may be as a result of higher Hb concentration obtained that was higher than the normal physiological values.

The mean corpuscular haemoglobin concentration measures the concentration of haemoglobin in a given volume of packed red blood cells. In this study there was no significant variation in the MCHC with age. This result was different from the findings obtained by Mmereole (2004; 2009) and Nyaulingo (2012) that the mean corpuscle haemoglobin concentration decreased as the age of birds increased.

Significant developmental changes of the birds were only observed for total white blood cell count and not in differential white blood cell count. WBC values were not within the normal physiological range ($4.07 - 4.32 \times 10^9/l$) of Wikivet (2012). White blood cell count values in chickens during the duration of the study indicated slight increase at week 1 and 2 of age. The differential white blood cell count values were not significantly different during the duration of the study and this was not consistent with the report of Tufan and Ramazan (2011) that leucocyte differentials were all significantly different in chick, young and adult pheasant.

In conclusion, significant age and body weight effect were observed for PCV, WBC, RBC, Hb, MCV and MCH for the duration of the study, indicating that majority of the haematological parameters for broilers increased with advancement of age. Also age of chickens had significant effect on their body weight. Younger chickens had reduced body weight, while older chickens had higher body weight. The result of this study is useful as a diagnostic tool in clinical evaluation of broilers and contributes baseline haematological values for management of chicks, young and adult broilers.

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