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Original research

Evaluations of the diurnal variations in the haematological profiles of domestic fowls

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ABSTRACT

The diurnal fluctuation in the haematological parameters of the domestic fowls was investigated using twenty four (24) male Isa Brown chickens. Twenty four (24) apparently healthy Isa Brown chickens of average mean weight 1.99±0.12 kg (1.85-2.30kg) aged 18 weeks were used for this study. The Isa Brown chickens (24) were randomly assigned to four treatment groups (A to D) of six chickens per group in a completely randomized design (CRD). Group A: Early morning (6am), Group B: Afternoon (12pm), Group C: Evening (6pm) and Group D: Mid Night (12am). Blood samples were collected from the birds at 6:00 am, 12:00 pm, 6:00 pm and 12:00 am, during a 12-hour light and a 12-hour dark period at a six hourly intervals. The mean haemoglobin (Hb) concentration (10.40±0.09g/dl) and packed cell volume (31.00±0.41%) at 12am were significantly (P < 0.05) higher than the other diurnal periods of the day. The mean red blood cell count (RBC) at early morning (6am) was significantly (P < 0.05) higher than the values at 12pm but showed no significant (p > 0.05) difference when compared to the values at 6pm and 12am. The mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) concentrations at 12pm were significantly (P < 0.05) higher when compared to the 6am values. The mean total leucocyte count (TLC), lymphocyte count and monocyte count at 6am were significantly (P < 0.05) higher than the values at 6pm. There was however no significantly (p > 0.05) variations in the mean corpuscular haemoglobin concentrations (MCHC), heterophil count, eosinophil count, basophil count and heterophil/lymphocyte (H/L) ratio among all the diurnal periods investigated. In conclusion, the haemoconcentration produced in this study might be responsible for the higher haematological parameters during the night (12am) and early morning (6am) because the birds were neither feeding nor drinking water at this period of the day

KeyWords: Diurnal variations, erythrogram, Isa brown, leucogram.

INTRODUCTION

Poultry production has been recognized as the cheapest source of animal protein in the developing countries (Ajayi, 2010; Abeyrathne *et al.*, 2013; Rahman *et al.*, 2013) and is also one of the most important sources of income and protein in developing countries (Zaman *et al.*, 2004).

The value of haematological parameters in the evaluation of the physical and health status of animals and birds (Hawky & Dennet, 1989; Zvorc *et al.*, 2006), the diagnosis, prognosis, treatment and prophylaxis of many livestock diseases (Hewett, 1974; Saror & Coles, 1975; Hawky & Dennet, 1989; Klinkon & Zadnik, 1999) has been well stressed. Haematological parameters have also been used to assess nutritional status of animals (Klinkon & Zadnik, 1999; Rekwot *et al.*, 1997) as well as conjuring metabolic features of particular cases (Kronfield, 1972). In poultry, they have also been demonstrated to be important indices of production and adaptability to prevailing environmental conditions (Olufemi & Fatunmbi, 1980; Mitchell &

MacLeod, 1983). The haematology of indigenous cattle and other livestock species and poultry has been well investigated (Saror & Coles, 1973; Saror & Coles, 1975; Oladele *et al.*, 2001a & b). According to Togun & Oseni (2005) haematological studies have been found useful for disease prognosis and for therapeutic and feed stress monitoring. It is often very difficult to assess the current health status of animals without detailed examination of blood (Amakiri et al., 2012). Examination of blood provides the opportunity to clinically investigate the presence of several metabolites and other constituents in the body as it plays a vital role in the physiological, nutritional and pathological status of the animal (Decuypere et al., 2001; Aderemi, 2004). Haematological parameters in birds have been shown to be influenced by various factors such as age, sex, season and nutrition. Packed cell volume (PCV), haemoglobin (Hb) concentration and red blood cell (RBC) count have been reported to increase with age in chickens (Islam et al., 2004), Pigeons (Columba livia forma domestica) (Pavlak et al., 2005), Turkeys (Oyewale & Ajibade, 1990) and Budgerigars (Melopsittacus undulatus) (Harper & Lowe, 1998). Packed cell volume (PCV) and Hb values have also been reported to be higher in males than in females in Turkeys (Oyewale & Ajibade, 1990) and Pigeons (Pavlak et al., 2005). Similarly, seasonal fluctuations in PCV and Hb concentrations have been reported in Pigeons in the tropics (Oladele et al., 2001b) and in the cold temperate region (Pavlak et al., 2005). Variations in the haematological parameters have also been reported in animals of the same age and sex, reared under the same conditions when sampled at different times of the day (Durotoye et al., 2000; Sanni et al., 2000). This significant but often neglected cause of variations in the haematological parameters is as a result of diurnal fluctuations or variations in these parameters following changes in daily physical and metabolic activities (Sanni et al., 2000; Piccione et al., 2001, 2005). Thus, according to Ferrer (1990), the time at which blood samples are collected must be considered before conclusions are drawn from the haematological data obtained to ascertain the physiological conditions in animals and man. The aim of this present study was therefore to determine the diurnal fluctuations in the haematological parameters of male domestic fowl (Isa Brown) in the hot humid tropics over a twenty four hour period.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

The study was carried out in the Poultry Unit of the Department of Animal Health and Production, College of Veterinary Medicine Teaching and Research Farm, Michael Okpara University of Agriculture, Umudike. Twenty four (24), 18-weeks old male apparently healthy commercial layer strain, of mean weight 1.99±0.12 kg (1.85-2.30kg) belonging to the Isa Brown chickens sourced from a poultry farm in the Southern Nigeria were used for this study. The

birds (chickens) were acclimatized for three weeks before the commencement of this study. Appropriate routine prophylactic medications were given as and when due or when necessary to ensure the optimal health of the experimental chickens. The birds were fed twice daily with commercial layers mash throughout the period of the study. Clean fresh cool water was provided *ad libitum* throughout the period of the study. Ethical approval was obtained from Ethical committee of College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria (MOUAU/CVM/REC/202216).

EXPERIMENTAL DESIGN

Twenty four (24) male Isa Brown chickens were randomly assigned to four treatment groups (A to D) according to the diurnal periods of six chickens per group in a completely randomized design (CRD). Group A: Early morning (6am), Group B: Afternoon (12pm), Group C: Evening (6pm) and Group D: Mid Night (12am). The birds were identified with tag letters thus; A₁₋₆, B₁₋₆, C₁₋₆ and D₁₋₆ representing groups A-D and they were kept individually in separate cages and were maintained in these separate cages throughout the period of the study. Two milliliters (2ml) of blood samples were collected from the wing veins of the experimental birds at 6:00 am, 12:00 pm, 6:00 pm and 12:00 am, during a 12hour light and a 12-hour dark period at a six hourly intervals and dispensed into sample bottles containing ethylene diamine tetraacetic acid (EDTA) at the ratio of blood to anticoagulant of 1-2 mg/ml and used to determine the red blood cell (RBC) and total leucocyte (TL) counts using Haemocytometer method (Schalm et al., 1975; Cheesbrough, 2006), packed cell volume (PCV) using Microhaematocrit method (Coles, 1986; Cheesbrough, 2006), Hb concentration using Cyanomethaemoglobin method (Kachmar, 1970; Brar et al., 2000; Cheesbrough, 2006) and Differential leucocyte counts (DLC) using the Leishman Staining Technique (Schalm et al., 1975; Cheesbrough, 2006). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) were calculated from the values obtained for RBC, PCV and Hb (Dacie & Lewis, 1984; Jain, 1986). Heterophil/lymphocyte (H/L) ratio was also determined by calculation by dividing heterophils with lymphocytes.

DATA ANALYSIS

The data collected for each of the haematological parameters during the diurnal phases were subjected to One Way Analysis of Variance (ANOVA) using statistical package for social sciences (SPSS) version 20.0. Variations in means were separated using Duncan's New Multiple Range Test (Steel & Torrie, 1980; SAS, 2010). Probability values < 0.05 were considered significant.

RESULTS

The mean haemoglobin (Hb) concentration and packed cell volume at 12am were significantly (P < 0.05) higher than the other diurnal periods. The mean red blood cell count (RBC) at early morning was significantly (P < 0.05) higher than the values at 12pm but showed no significant (p > 0.05) difference when compared to the values at 6pm and 12am. The mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) concentrations at 12pm were significantly (P < 0.05) higher when compared to the 6am values. The mean total leucocyte count (TLC), lymphocyte count and monocyte count at 6am were significantly (P < 0.05) higher than the values at 6pm. There was however no significantly ((p > 0.05)) variations in the mean corpuscular haemoglobin concentrations (MCHC), heterophil count, eosinophil count, basophil count and heterophil/lymphocyte

(H/L) ratio among all the diurnal periods investigated in this study.

DISCUSSION

The results of the diurnal variations in the haematological profiles are presented in tables 1 and 2. The PCV in the present study showed considerable daily variations. The PCV was highest at 12:00 am, but dropped considerably at 06:00 pm. This decline may be as a result of haemodilution following increased feed and water consumption by the birds at this period of the day. Our findings was in contrast to the slight increase in the PCV at 2:00 pm reported by Snow & Martin (1990) probably due to an increase in the number of circulating red blood cells, following splenic contraction as a result of increased physical and metabolic activities. Our finding was also in contrast to the slight increase in the PCV

Table 1: The mean erythrogram of Isa Brown chickens during the diurnal periods. Values are expressed as means \pm SEM in the table

Parameters	Treatments				
	6:00 am	12:00 pm	6:00 pm	12:00 am	
Hb (g/dl)	8.73 ± 0.27^{a}	8.18±0.21 ^a	$9.50{\pm}0.18^{b}$	10.40±0.09 ^c	
PCV (%)	$26.25{\pm}0.75^{ab}$	24.25 ± 0.63^{a}	$27.75{\pm}0.75^{\text{b}}$	$31.00 \pm 0.41^{\circ}$	
RBC (x10 ¹² /L)	2.35±0.23 ^b	1.50±0.17 ^a	2.13 ± 0.14^{b}	2.13±0.10 ^b	
MCV (fl)	$115.39{\pm}12.27^{a}$	167.62 ± 18.48^{b}	$131.94{\pm}8.79^{ab}$	$146.42{\pm}6.45^{ab}$	
MCH (pg)	38.30 ± 3.89^{a}	$56.57{\pm}6.35^{b}$	$45.31{\pm}3.51^{ab}$	$49.13 {\pm} 2.10^{ab}$	
MCHC (g/dl)	33.24±0.37	33.72±0.35	34.31±1.14	33.56±0.22	

abcMean values in the same row with no similar superscripts are significantly different (P<0.05)

Table 2: The mean total Leucocyte count, Differential Leucocyte counts andHeterophil:Lymphocyte (H/L) ratio of Isa Brown chickens during the diurnalperiods. Values are expressed as means ± SEM in the table

Parameters	Treatments				
	6:00 am	12:00 pm	6:00 pm	12:00 am	
TWBC (×10 ⁹ /L)	14.36±5.58 ^b	6.73±3.14 ^{ab}	1.71±0.63 ^a	2.44±0.29 ^a	
Heterophil (×10 ⁹ /L)	7.95±4.39	3.80±2.13	0.95±0.40	1.12±0.21	
Lymphocyte $(\times 10^{9}/L)$	5.94±1.78 ^b	2.59±0.91 ^a	$0.52{\pm}0.20^{a}$	1.18±0.10 ^a	
Monocyte (×10 ⁹ /L)	0.32±0.17 ^b	0.02±0.02 ^a	0.01 ± 0.00^{a}	0.03±0.01 ^a	
Eosinophil (×10 ⁹ /L)	0.08±0.07	0.02±0.02	0.01±0.01	0.00 ± 0.00	
Basophil (×10 ⁹ /L)	0.08±0.07	0.31±0.12	0.23±0.08	0.12±0.03	
H/L Ratio	1.34±0.53	1.23±0.26	1.95±0.66	0.96±0.18	

^{ab}Mean values in the same row with no similar superscripts are significantly different (P<0.05)

at 12:00 pm reported by Oyewale & Olowookorun (1986) in WAD goats and this was attributed the probably to increased metabolic activities and oxygen consumption at that period. Our finding was equally in contrast to the increase in the PCV at 6:00am reported by Azeez et al. (2009) in indigenous chickens possibly due to haemoconcentration probably because the birds were yet to be offered feed and water as it was still very early (6:00am) to do so. In this present study, PCV was highest at 12:00am when the birds were in the dark-phase of the day and were no longer feeding and drinking water. Similarly Piccione et al. (2005) reported an increase in PCV at night in horses.

The lower Hb, PCV and RBC in this study at 12:00pm than at any other period of the day may likely be as a result of haemodilution following increased feed and water consumption at this period of the day as was observed by Jain (1986). The MCV increased at this period which is an indication of a fall in plasma osmotic pressure following haemodilution after feed and water consumption by the birds. In contrast, higher values of these parameters (RBC, Hb, MCH and MCHC) were recorded at night (12:00 am) and early morning (6:00 am). This might be probably as a result of haemoconcentration because the birds were neither feeding nor drinking water at this dark phase of the day despite the ongoing or continuous metabolic activities. However, Maxwell *et al.* (1990) reported an increase in the RBC count while PCV, Hb, MCH and MCHC were reduced in feed restricted broilers and this report agrees with our findings.

Sanni *et al.* (2000) also reported a progressive increase in the RBC count of the African giant rats (*Cricetomys gambianus*), which were nocturnal animals, the lowest value obtained at 12:00pm noon while the highest value was obtained at 12 midnight and this agrees with our findings.

The PCV and MCV values of the giant rats were however reported by these authors to be higher during the day than in the night. The pattern of diurnal variation observed in the present study for PCV and Hb are in agreement with our findings from the pattern observed in humans (Touitou *et al*, 1986, Pocock, *et al*, 1989). These authors observed a slightly declining trend in these parameters throughout the day, and suggested that this was due to the circadian variation in plasma volume, or to activity. Rehder *et al.* (1982) also observed a decline in the values of the PCV and RBC in captured red hawk (*Buteo jamaicensis*) over a 12-hour period. They found the highest value in the morning at 9:00 am and the least value at 9:00 pm and this report disagrees with our findings.

The total leucocyte count (TLC) at early morning (6:00 am) in this study was higher than any other periods of the day leading to a physiological leukocytosis which usually progresses to lymphocytosis and this could be because of haemoconcentration as the birds were yet to receive feed and water as it was still very early for the poultry attendant to do so. We observed a significant reduction in the lymphocytes at 6:00 pm following feed and water intake. Human studies suggest that this phenomenon is due to the distribution and function of immune cells, and decreased lymphocytes and monocytes observed in humans after feeding reflect migration of these cells to lymphoid tissues to support primary immune responses in the gut (Hansen et al., 1997; Van Oostrom et al., 2002, 2003; Cheng et al., 2010; Lippi et al., 2010). The Hb value obtained in the present study (9.20±0.19g/dl) was similar to that of 8.9-9.8 g/dl, reported in broiler chickens reared under tropical conditions (Onibi et al., 2011). The total leucocyte count (TLC) of 6.31 \pm 2.41 \times 10^{9} /L, recorded in Isa Brown chickens in the present study was significantly higher than the count of $2.2 - 7.5 \times 10^{9}$ /L obtained in broilers (Adeyemo & Sani, 2013), but lower than the mean TLC of 24.60×10^9 /L, recorded by Durotoye *et al*. (2000) in indigenous chickens. The pattern of diurnal fluctuation of WBC count in broilers in the study of Makeri et al. (2017) agrees with our findings but contrary to the finding of Piccione et al. (2005) who reported no significant daily diurnal variations in the total leucocyte count (TLC) of horses.

CONCLUSION

Diurnal fluctuations cause statistically significant alterations on the erythrogram, leukogram and H/L ratio of domestic chickens in this study. However, for healthy Isa Brown chickens, these changes do not appear to be clinically significant, but for sick ones with borderline values, they may be clinically relevant and calls for urgent concerns.

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