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Diurnal Fluctuation in Haematological Parameters of the Domestic Fowl in the Hot Humid Tropics

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Abstract: Diurnal fluctuation in haematological parameters such as packed cell volume (PCV), red blood cell (RBC) count, haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and erythrocyte osmotic fragility of the domestic fowl in the hot humid tropics was investigated using Nera Black cocks. Blood samples were collected from the birds at 6:00 am, 10:00 am, 2:00 pm, 6:00 pm, 10:00 pm and 2:00 am during a 12-hour light and a 12-hour dark period. PCV showed considerable diurnal variation with the lowest value obtained at 10:00 am and the peak value recorded during the early morning (2:00 am). RBC, Hb, MCH and MCHC values also varied according to the time of the day, with the lowest values observed at 2:00 pm, probably as a result of haemodilution following increased feed and water consumption at this period of the day. Peak values for RBC, Hb, MCH and MCHC were observed at 10:00 pm when the birds were already roosting (during the dark phase of the day) as a result of which physical and metabolic activities were generally lowered. Haemoconcentration so produced might be responsible for the higher haematological parameters during the night because the birds were neither eating nor drinking water at this period of the day. Erythrocyte osmotic fragility at 0.3% NaCl concentration was also significantly higher ($P < 0.05$) at 6:00 am than at any other period of the day.

Key words: Diurnal fluctuation, haematological parameters, domestic fowl

INTRODUCTION

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 and Ajibade, 1990) and budgerigars (*Melopsittacus undulatus*) (Harper and Lowe, 1998). PCV and Hb values have also been reported to be higher in males than in females in turkeys (Oyewale and Ajibade, 1990) and pigeons (Pavlak *et al.*, 2005). Similarly, seasonal fluctuations in PCV and Hb concentration have been reported in pigeons in the tropics (Oladele *et al.*, 2001) and in the cold temperate region (Pavlak *et al.*, 2005). Variations in 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 haematological parameters is as a result of diurnal fluctuation or variation 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 physiological conditions or otherwise in man and animals.

This present study was aimed at determining the fluctuations, if any, in the haematological parameters of the domestic fowl in the hot humid tropics over a twenty four-hour period.

MATERIALS AND METHODS

Twenty-one week old male domestic fowls of the Nera black strain were used. The birds belong to a commercial layer strain bred specifically for egg and meat production in Nigeria. Blood samples were collected from the birds into heparinized tubes at 4 hourly intervals (i.e. 6:00 am, 10:00 am, 2:00 pm, 6:00 pm, 10:00 pm and 2:00 am) during a 12-hour light and a 12-hour dark period. The samples were taken from the jugular vein of each of 6 groups of birds consisting of 5 birds per group at each of the periods.

The red blood cell (RBC) count was determined by the haemocytometer method, the packed cell volume (PCV) by the microhaematocrit method and the haemoglobin (Hb) concentration by the cyanmethaemoglobin method. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the values obtained for RBC, PCV and Hb according to Jain (1986).

Erythrocyte osmotic fragility was determined according

to the method described by Oyewale (1992). Briefly, 0.02 ml of blood was added to tubes containing increasing concentration of phosphate-buffered sodium chloride (NaCl) solution at pH 7.4 (0, 0.1, 0.2, 0.3, 0.5, 0.7, 0.8 and 0.9%). The tubes were gently mixed and incubated at room temperature (29°C) for 30 minutes. The content in each tube was then centrifuged at 1500 rev/min for 10 minutes and supernatant decanted. Optical density of the supernatant was determined spectrophotometrically at 540 nm using SM 22 PC Spectrophotometer (Surgienfield Instruments, England). Haemolysis in each tube was expressed as a percentage, taking haemolysis in distilled water (0% NaCl) as 100%.

All results obtained were statistically analyzed for significance using one-way ANOVA in Graph pad Prism version 4.00, April, 2003 statistical software. Results are expressed as mean±SEM while $P < 0.05$ was considered to be significant.

RESULTS

As shown in Fig. 1, the PCV of the fowl was significantly higher ($P < 0.05$) at 6:00 am (31.20±1.83%) than at 10:00 am (25.75±0.63%) or 6:00 pm (26.20±1.11%). The PCV obtained at 10:00 pm (31.00±1.47%) was also higher than at 10:00 am ($P < 0.01$) or 6:00 am ($P < 0.001$). In addition, the value at 2:00 am (31.40±0.68%) was significantly higher than PCV at 10:00 am ($P < 0.001$) or 2:00 pm ($P < 0.05$). It was also higher than the PCV at 6:00 pm ($P < 0.01$).

As illustrated in Fig. 2, the RBC count also varied according to the time of the day. The lowest RBC count (1.46±0.14 x 10¹²/L) was obtained at 2:00 pm. It was significantly lower ($P < 0.01$) than the RBC count obtained at 10:00 am (1.58±0.06 x 10¹²/L) or 6:00 pm (2.03±0.04 x 10¹²/L). Similarly, RBC count at 2:00 pm was lower than the count (1.96±0.07 x 10¹²/L) at 10:00 pm ($P < 0.05$) or the value (1.93±0.06 x 10¹²/L) obtained at 2:00 am ($P < 0.01$).

Figure 3 shows the daily fluctuations in haemoglobin (Hb) concentrations in the fowl. The Hb concentration at 6:00 am (8.30±2.07 g/dl) was significantly higher ($P < 0.05$) than the value at 10:00 am (7.83±0.58 g/dl) or 2:00 pm (5.52±0.41 g/dl). The Hb value at 6:00 pm (7.78±0.35 g/dl) was also higher ($P < 0.0001$) than that obtained at 10:00 am. Similarly, the Hb concentration at 10:00 pm (7.33±0.88 g/dl) or 2:00 am (8.24±0.47 g/dl) was higher ($P < 0.01$) than the value at 10:00 am. However, the Hb value at 2:00 pm was significantly lower ($p < 0.01$) than the value at 6:00 pm, 10:00 pm or 2:00 am.

Figure 4 shows the MCV values of the fowl at the different time of the day. The value of MCV (191.15±13.21fl) was highest at 2:00 pm, being significantly higher than that at 6:00 am (155.71±10.66fl) ($P < 0.05$) or 6:00 pm (129.04±5.55fl) ($P < 0.01$). The MCV at 2:00 pm was also higher ($P < 0.01$) than the value at 10:00 pm (158.73±8.27 fl). The MCV value which

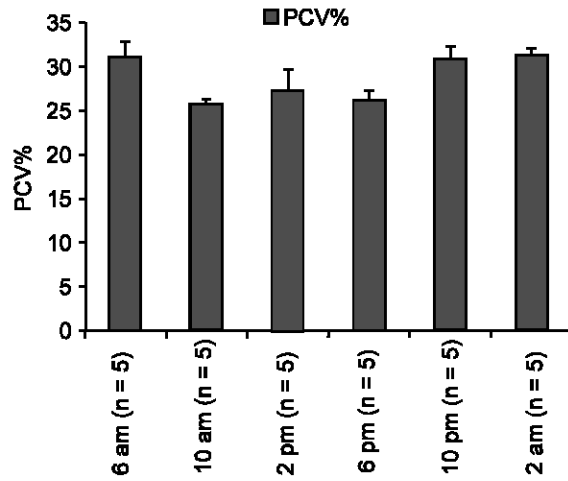


Fig. 1: Packed cell volume (PCV) of the domestic fowl at different times of the day. Values are means and vertical bars represent SEM. n = number of birds.

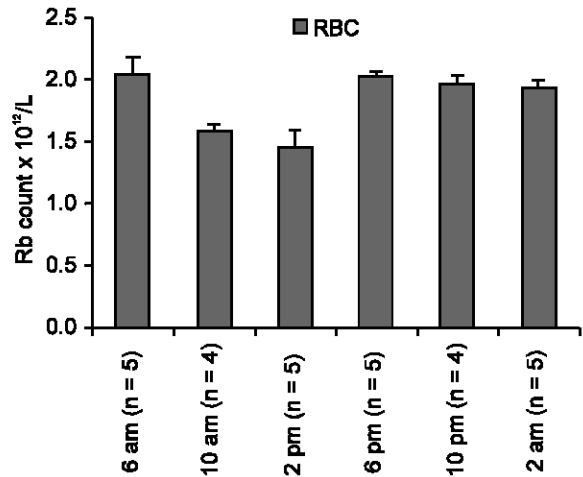


Fig. 2: Red blood cell (RBC) count of the domestic fowl at different times of the day. Values are means and vertical bars represent SEM. n = number of birds.

was obtained at 6:00 pm was significantly lower than the value at either 6:00 am (155.71±10.66 fl) ($P < 0.05$) or 10:00 am (163.9±5.21 fl) ($p < 0.01$). Also, the MCV at 6:00 pm was lower ($p < 0.01$) than each of the values obtained at 2:00 pm (191.15±13.21 fl), 10:00 pm (158.73±8.27 fl) and 2:00 am (163.17±7.24 fl).

As shown in Fig. 5, the MCH was higher ($P < 0.05$) at 10:00 am (51.11±3.13 pg) than the MCH values at 2:00 pm (38.25±2.14 pg) or 6:00 pm (38.35±1.90 pg). It was also higher than the MCH value of (37.18±3.05 pg) obtained at 10:00 pm ($P < 0.05$). As illustrated in Fig. 6, the MCHC also varied according to the time at samples

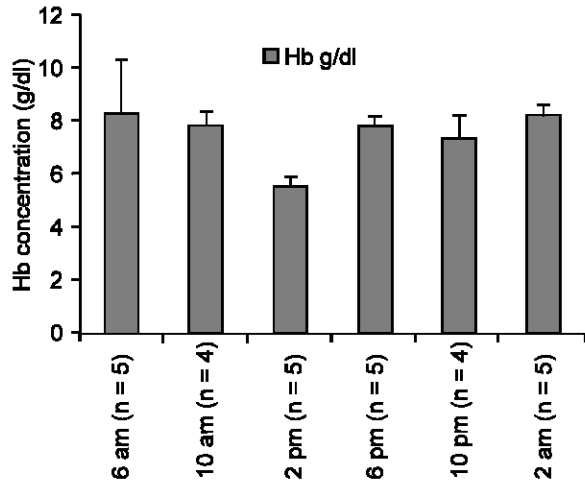


Fig. 3: Haemoglobin (Hb) concentration of the domestic fowl at different times of the day. Values are means and vertical bars represent SEM. n = number of birds.

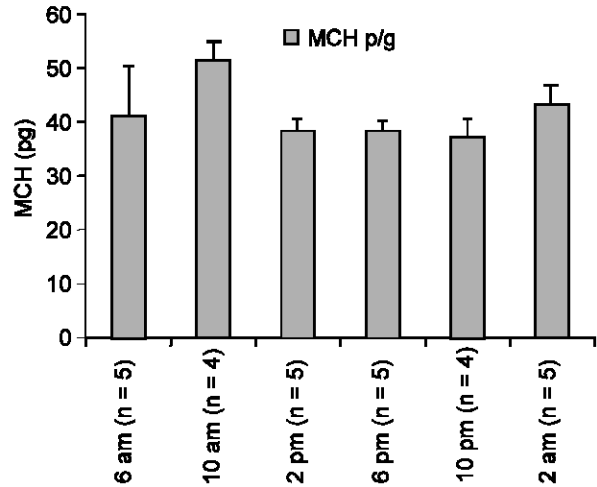


Fig. 5: Mean corpuscular haemoglobin (MCH) of the domestic fowl at different times of the day. Values are means and vertical bars represent SEM. n = number of birds.

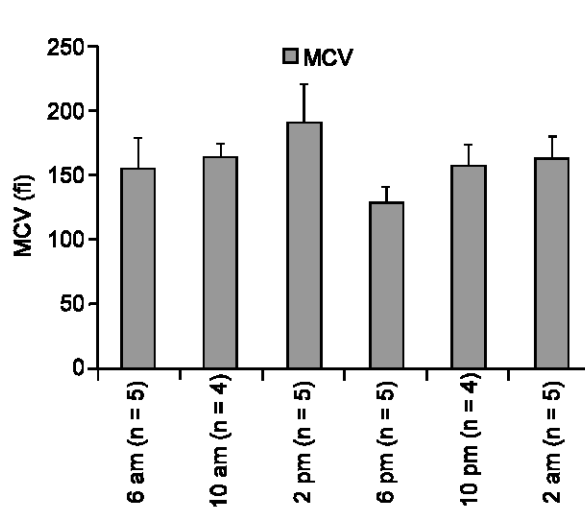


Fig. 4: Mean corpuscular volume (MCV) of erythrocytes in the domestic fowl at different times of the day. Values are means and vertical bars represent SD. n=number of birds.

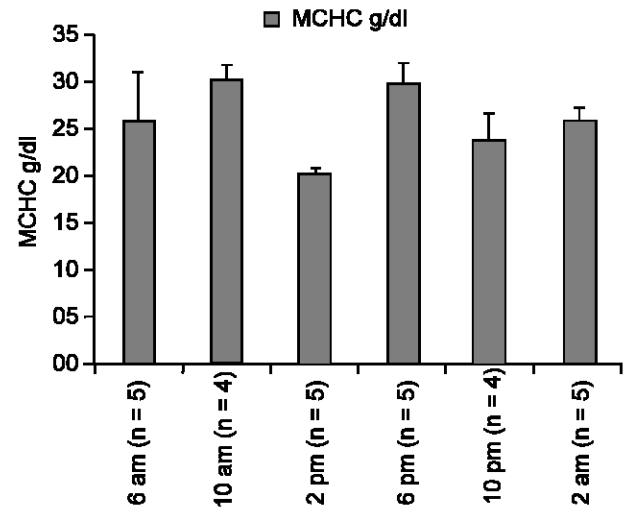


Fig. 6: Mean corpuscular haemoglobin concentration (MCHC) of the domestic fowl at different times of the day. Values are means and vertical bars represent SEM. n = number of birds.

were collected, the lowest value (20.12 ± 0.74 g/dl) occurred at 2:00 pm. It was significantly lower ($P < 0.001$) than MCHC values at 10:00 am (30.28 ± 1.61 g/dl), 6:00 pm (29.97 ± 2.04 g/dl) and 10:00 pm (23.79 ± 2.85 g/dl).

Figure 7 shows the osmotic fragility of erythrocytes of the domestic fowl at the different time periods of the day. Except at the 0.3% NaCl concentration, there were no significant differences in the fragility of erythrocytes between the different time periods at the various NaCl concentrations. At the 0.3% NaCl concentration however, the fragility of erythrocytes was higher ($P < 0.05$) at 6:00

am than at 10:00 am or 2:00 pm. Also, the fragility at 6:00 am was higher ($P < 0.05$) than the value at 6:00 pm.

DISCUSSION

The PCV in the present study showed considerable daily fluctuations. The PCV was high at 2:00 am, but dropped considerably at 10:00 am. This decline may be as a result of haemodilution following increased feed and water consumption by the birds at this period of the day. A slight increase in the PCV was also observed at 2:00 pm, probably due to an increase in the number of circulating red blood cells, following splenic contraction

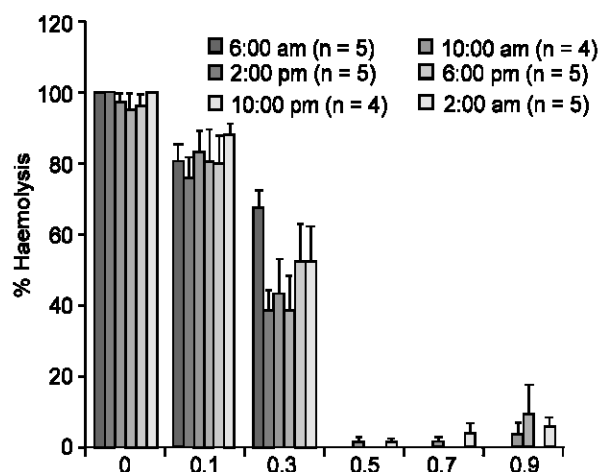


Fig. 7: Osmotic fragility of fowl erythrocytes at different time of the day. Values are means and vertical bars show SEM.

as a result of increased physical and metabolic activities (Snow and Martin, 1990). Similar increase in PCV was observed at 12 noon in West African dwarf goat by Oyewale and Olowookorun (1986). This was attributed to increased metabolic activities and oxygen consumption at this period. In the present study, the highest PCV was obtained at 10:00 pm when the birds were in the dark-phase of the day and were no longer feeding nor drinking. Piccione *et al.* (2005) observed an increase in PCV at night in horses. The PCV of $25.75 \pm 0.63\%$ at 10:00 am in the present study is lower than the value of $28.29 \pm 2.16\%$ reported by Oyewale and Durotoye (1988) in Hubbard fowls in the same hot humid tropics. This difference could be as a result of the genetic difference between the birds, since Nera black fowls were used in the present study and Hubbard fowls were used by Oyewale and Durotoye (1988).

The lower RBC, Hb, MCH and MCHC at 2:00 pm 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 observed by Jain (1986). The MCV increased at this period, 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 obtained at night (10:00 pm) and early morning (2:00 am). This might not be unconnected with haemoconcentration because the birds were neither eating nor drinking at this dark phase of the day despite the ongoing or continuous metabolic activities. Maxwell *et al.* (1990) reported an increase in RBC count, while PCV, Hb, MCH and MCHC were reduced in feed restricted broilers. Sanni *et al.* (2000) also reported a progressive increase in RBC count of the African giant rat (*Cricetomys gambianus*), which were nocturnal animals, the lowest value obtained at 12

noon while the highest value was obtained at 12 midnight. PCV and MCV values of the giant rats were however reported by these authors to be higher during the day than in the night. Pocock *et al.* (1989) in a study in man observed a weak decline in PCV, RBC and Hb values in man during the day with a direct relationship with the feeding habit and the plasma volume. 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. In the present study, the erythrocyte osmotic fragility was increased at 0.3% NaCl concentration in birds sampled at 6:00 am compared with any other time. This may be due to haemoconcentration at 6:00 am when the birds had not had access to feed nor water. Yaqil *et al.* (1976) had also reported an increase in osmotic fragility in water-deprived chicken as a result of haemoconcentration. In contrast, Mafuvadze *et al.* (2008) did not observe any change in erythrocyte osmotic fragility of the guinea fowl that were deprived of water for up to 48 h. The increase in erythrocyte fragility observed in the present study could also have been due to a number of other factors such as oxidative damage to the polyunsaturated fatty acids in the erythrocyte membrane as observed by Droge (2002). Sen (1995) also associated increased osmotic fragility with increased fluidity of the erythrocyte membrane due to lipid peroxidation. Thus, the high erythrocyte osmotic fragility observed at 6:00 am may probably be the result of increased lipid peroxidation of the erythrocyte membrane caused by haemoconcentration. This is in agreement with the report of Jain (1986), who observed a positive correlation between haemoconcentration and erythrocytes osmotic fragility in cattle.

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