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DIURNAL VARIATION IN BLOOD PARAMETERS IN THE CHICKEN IN THE HOT TROPICAL CLIMATE.

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Twelve adult male chicken of the Nigerian local strain were bled every 3 Hours for 24 hours. Haematological and serum biochemical parameters were measured in the samples collected. Variations in the levels of these parameters throughout the 24 hours were determined. Thirteen out of the parameters measured showed significant diurnal variations. These include RBC, PCV, Hb, MVC, WBC, Na^+, K^+, urea, cholesterol, triglycerides, ALP, GPT and GGT.

Key words: *Diurnal variation; blood picture; chicken.*

The homeostatic or "steady state" picture of an organism is an oversimplification. This is clear whenever a variable is measured over a protracted period of time. Under such situations, physiological variables are not constant, although they remain within a narrow range. They exhibit rhythmic changes (Minors and Waterhouse, 1986). Available evidence has shown that these rhythms are under the constant control of some endogenous oscillators (Cloudley-thompson, 1980). These endogenous oscillators are themselves influenced by other factors such as exogenous oscillators like photoperiod, activity pattern, geographical location, social cues etc (Aschoff *et al*, 1971, Binkley, 1982).

Effects of rhythms like circadian/diurnal, seasonal, annual etc, on blood parameters have been studied in humans (Markowitz *et al* 1981), monkeys, (Klein *et al*, 1985), Laboratory rodents (Berger, 1980) and chicken (Twiest and Smith, 1970).

The influence of biorhythms on physiological parameters in not only affected by species, age and sex, but also by climate and geographical location etc (Mills et at, 1978).

It is therefore important to determine the influence of biorhythms on the physiological parameters in all species and in all localities. Results of such studies especially on the blood system will serve a useful tool in clinical and experimental haematology.

Previous studies on the haematology of the Nigerian domestic chicken (Makinde *et al*, 1986, Oyewale, 1987, Oyewale and Durotoye, 1988) did not consider the factor of biorhythm. It is therefore the objective of this study to examine the variations in blood parameters which occur during the 24 hour period of the day in the chicken under a tropical climate like that of Nigeria.

MATERIALS AND METHODS

Experimental animals

Twelve adult male local strain of domestic chicken (*Gallus domesticus*) were used in this study. They were purchased from a local market in Ibadan, Nigeria, and kept in two communal housing unit (6 birds per unit), measuring 1.5 x 1.5 x 0.3 m. The birds were treated with anti-stress vitamins (Trisol, Frank Wright Ltd, U.K.), and anti-coccidiosis (Embazine Forte, Rhone Poulence, U.K.). Birds were allowed 14 days to adapt to the new environment and were fed commercially-prepared adult poultry feed (Ladokun Feeds Ltd, Ibadan, Nigeria), and water *ad-libitum*.

Blood sampling

Bloods sampling was done 3-hourly, beginning from, 03.00 hr of day 1, and ending at 03.00 hr. of day 2. During the period of darkness, blood collection was done under a deep red light. About 2 ml of blood was collected through the jugular vein from each bird, 0.5 ml of which was dispensed into clean bijou bottles containing anticoagulant EDTA. The rest was allowed to clot. The anti-coagulated blood was used to determine red blood cell (RBC) count, packed cell volume (PCV), haemoglobin (Hb) concentration, and white blood cell (WBC) count. Serum was separated from the clotted blood following centrifugation at 3,000 r.p.m. for 10 minutes at room (28 C), and stored at -20 C until required for other tests.

Experimental procedure

Red and white blood cells were counted using the haemocytometer method, PCV by the microhaematocrit method, and Hb concentration by the cyanmethaemoglobin method. Mean corpuscular volume (MCV), MCH, and MCHC were calculated from the above as described by Schalm *et al*, (1975). Serum sodium, potassium and calcium were determined with a flame photometer (Coring, Model 410, U.K.). Chloride and bicarbonate ions were measured by the mercuric nitrate method of Schales and Schales (1941). A modification of phosphomolybdic method was used to assess the concentration of phosphate ions, while serum proteins were measured using the hand refractometric method.

Cholesterol was determined as described by Cole (1986), while serum triglyceride, urea and creatinine were determined using the standard Technicon methods on the SMA 12/60 analyser. Serum enzymes glutamate pyruvate transaminase (GPT) and glutamate oxalate transaminase (GOT) were converted to oxoacids and then coupled to 2, 4, dinitrohenyl-hdrazine forming hydrazone, the intensity of the colour of which depends on the quantity of enzyme present (Toro and Ackermannn, 1975). The enzyme alkaline phosphatase and ALP were measured by a reaction which terminated in colour development measured at 500 nm wavelength. GGT was measured by adding the rehydrated substrate to the sample at 30 C for 2-3 minutes. The absorbance of the resulting solution was measured at 405 nm wavelength (King and Armstrong, 1934).

Statistics

Means were taken for the data obtained at each sampling time, and subjected to statistical test of significance using the ANOVA repeated measures.

RESULTS

Red blood cell count, PCV and Hb concentration all varied in a similar pattern, each attaining a peak around 18.00 hr (Fig 1).

As shown in table 1, mean value of RBC between 12.00 - 18.00 hr is significantly ($P<0.002$) higher than it is between 21.00 and 03.00 hr. Between 21.00 and 03.00 hr, the mean value of PCV was 34.22 ± 1.6 %. This is significantly ($P<0.05$) higher than the value between 03.00 and 09.00 hr. The Hb concentration of 11.33 ± 0.29 mg/dl between 12.00 and 18.00 hr is significantly ($P<0.02$) higher than it was between 21.00 and o3.00 hr i.e., 11.01 ± 0.23 mg/dl.

Mean values of MCV, MCH and MCHC were lowest between the hours of 03.00 and 09.00 hr, and highest during 21.00 and 03.00 hr. Although variations in the level of MCH and MCHC throughout the day were not significant, that of MCV was significantly ($P<0.02$) lower between 03.00 and 09.00 hr, than between 12.00- 18.00 hr and 21.00-03.00.00 hr respectively.

White blood cells peak value ($27.4 \pm 3.9 \times 10^9/1$) occurred between 21.00- 03.00 hr, and the lowest value ($22.9 \pm 2.9 \times 10^9/1$) occurred during the period of 12.00-18.00 hr. A significant ($P<0.01$) difference exists between these figures.

Serum Na^+ and K^+ ion concentrations also varied significantly ($P<0.01$) for Na^+) and ($P<0.05$ for K^+) throughout the 24 hour period. The other serum electrolytes studied (Ca^{++}, $C1^-$, HCO_3^- PO_4^-), did not vary significantly throughout the 24 hours of the day

Table 2 shows the mean values for protein, urea, cholesterol and some serum enzymes. No significant variation was observed for total protein, albumin or globulin.

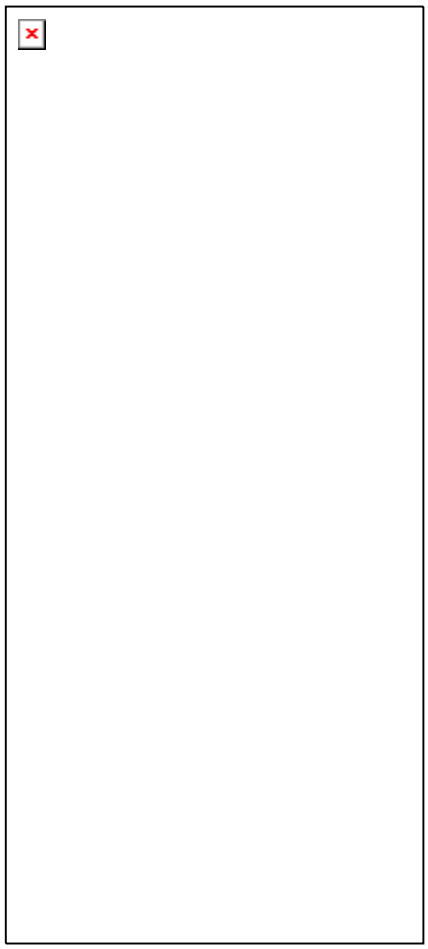

Figure 1.
Daily Variations in erythrocyte count, haematocrit and Haemoglobin concentration.

Table 1
Diurnal variation in some haematological parameters and serum electrolytes in the Nigerian local chicken during the 24 hr period of the day.

Parameters	03.00-09.00hr	12.00 - 18.00 hr	21.00 - 03.00 hr
RBC (1012/I)	2.21 ± 0.10	2.48 ± 0.20e	2.18 ± 0.21
PCV (%)	32.25 ± 2.80	33.3 ± 3.80	34.22 ± 1.60b
Hb (Mg/dl)	11.05 ± 0.23	11.33 ± 0.29c	11.01 ± 0.23
MCV (FI)	136.80 ± 3.40	141.50 ± 8.60c	142.80 ± 6.90c
MCH (Pg)	29.00 ± 1.70	29.60 ± 2.10	29.60 ± 3.7
MCHC (G/dl)	30.13 ± 3.10	30.90 ± 1.70	31.80 ± 2.50
WBC (10^9/I)	25.70 ± 3.20b	22.90 ± 2.90	27.40 ± 3.90d
Na^+ (MmoI/I	139.90 ± 3.40d	134.90 ± 4.20	135.8 ± 4.9
K+ (MmoI/I	4.10 ± 0.50	4.70 ± 0.7b	4.3 ± 0.7
Ca++ (MmoI)	8.50 ± 0.90	8.70 ± 1.0	8.5 ± 1.0
CI- (MmoI/I)	102.20 ± 10.8	101.70 ± 15.1	102.2 ± 12.3
HCO3- (MmoI/I)	22.80 ± 3.80	22.40 ± 7.3	22.5 ± 4.5
PO4- (MmoI/I)	4.10 ± 0.30	4.30 ± 0.6	3.9 ± 0.4

Figures with superscrip on the same row are significantly different.
b = P<0.05; c = P<0.02; d = P<0.01; e P<0.002

Table 2: Variations in the concentrations of serum biochemical parameters during the 24 hour period of the day in the Nigerian male chicken.

Parameters	03.00–09.00hr	12.00–18.00	21.00 – 03.00
Total protein (mg/dl)	6.2 ± 0.1	6.2 ± 0.1	6.3 ± 0.2
Albumin (mg/dl)	2.97 ± 0.15	3.10 ± 0.60	3.01 ± 0.50
Globulin (mg/dl)	3.25 ± 0.15	3.16 ± 0.45	3.17 ± 0.42
Urea (mg/dl)	22.4 ± 2.9	24.6 ± 2.1d	21.7 ± 1.6
Cholesterol (mg/dl)	111.0 ± 9.5	113.3± 10.1b	104.6 ± 8.7
Triglyceride(mg/dl)	77.5 ± 6.2	79.8 ± 5.9c	72.7 ± 4.5
Creatinine (mg/dl)	0.96 ± 0.10	1.05 ± 0.3	1.00 ± 0.18
ALP (IU/L)	158.4 ± 12.3	164.8 ± 23.5	168.5 ± 10.4d
GOT (IU/L)	33.0 ± 2.5	30.3 ± 1.9	31.4 ± 2.4
GPT (IU/L)	23.1 ± 1.7c	18.8 ± 2.5	22.2 ± 3.7c
GGT (IU/L)	9.7 ± 1.8d	6.5 ± 1.2	5.9 ± 1.2

Figures carrying superscript on the same row are significantly different.
b= P<0.05; c = P<0.01; d = P<0.002.

For urea, the 24.6 ± 2.1 mg/dl value during the hours of 12.00-18.00 hr is significantly (P<0.002) higher than the value at 21.00 - 03.00 hr. Peak mean values for cholesterol, triglycerides and creatinine were observed between the period of 12.00 - 18.00 hr. While fluctuations in the concentration of creatinine throughout the day is not significant, the values of cholesterol (113.3 ± 10.1), and triglycerides (79.8 ± 5.9) mmos/1 are respectively significantly (P<0.05) and (P<0.01) higher than at other times during the day (Table 2).

Among the enzymes measured, only GOT did not vary significantly throughout the 24 hours of the day. Between 21.00 and 03.00 hr, the 168.5 ± 10.4 mg/dl value of ALP is significantly (P<0.002) higher than the value between 03.00 - 09.00 hr. The value of GPT at 12.00 - 18.00 hr is significantly (P<0.01) lower than during any other time of the day. The concentration of GGT enzyme was 9.7 ± 1.8 IU/L between 03.00 - 09.00 hr. This is significantly (P<0.002) higher than corresponding values between 12.00 - 18.00 hr. and 21.0 - 03.00 hr.
Table 3 shows the 24 hr mean values of some of the parameters determined in this study as they compare with similar figures from different studies.

DISCUSSION

In this study, 21 blood parameters were determined including 4 serum enzymes in the blood samples collected from 12 male, adult Nigerian chicken, while 3 others were calculated. Thirteen of the parameters (RBC, PCV, Hb, MCV, WBC, Na^+, K^+, urea, cholesterol, triglycerides, ALP, GPT, and GGT) showed significant diurnal variation, while the rest did not.
Similar findings have been shown in the temperate climate in chicken (Twiest and Smith, 1970), and in the eagle owl (Garcia-Rodriguez, *et al*, 1987).

Table 3: Twenty four hour mean values of some hematological and serum biochemical parameters in the Nigerian male chicken.

Serum Parameters	24– hour mean (±SD
Red Blood Cell count (X 10^{12}/I)	2.29 ± 0.37*
Packed Cell Volume (%)	35.60 ± 4.30*
Haemoglobin Concentration (mg/dl)	11.40 ± 2.75*
White Blood Cell count (X 10^{9}/I)	24.62 ± 3.57*
Na^{+} (Mmol/I)	135.85 ± 3.65
K^{+} (Mmol/I)	4.38 ± 0.36
HCO_3^{-} (Mmol/I)	22.57 ± 1.67
PO_4^{-} (Mmol/I)	4.14 ± 0.26
Total Protein (Mg/dl)	6.18 ± 0.34*
Urea (Mg/dl)	22.9 ± 2.34
Cholesterol (Mg/dl)	104.7 ± 11.54
Creatinine (Mg/dl)	1.00 ± 0.11
GOT (IU/L)	31.56 ± 2.12
GPT (IU/L)	21.37 ± 3.10
GGT (IU/L)	7.36 ± 0.30

Twelve (12) birds were used and each animal bled nine times. Each datum represents a mean of 108 data. Data with asterisks are in good agreement with similar data previously

The pattern of diurnal variation observed in the present study for RBC, PCV and Hb are different from the pattern observed in humans (Toutou *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. Activity is probably not responsible for the pattern observed in the present study, as the local chicken remain active from morning till about 1800 hr, after they start to prepare to roost.

The opposing trend in the diurnal variation between the WBC on one hand, and RBC, PCV and Hb on the other, observed in the present study had been reported earlier in other species (Pocock *et al*, 1986). The importance of this is not immediately obvious and may therefore not be of any physiological significance. In the study by McPherson *et al*, (1978), and Minors and Waterhouse, (1981), using human subjects, only serum potassium showed diurnal variation among the serum cations. In the present study, significant diurnal variation was observed for both sodium and potassium. This is similar to the result of the study by Berezkin *et al.*, (1988) in which significant diurnal variation in serum concentration of sodium and potassium was demonstrated, especially during winter. Our observation in the present study concerning serum calcium and phosphorus is in support of earlier study by Garcia-Rodriguez *et al*, (1987) who did not observed any significant diurnal variation in the serum levels of these parameters. A continuous rise in serum PO_4^{-} throughout the day has however been reported in man, and linked to the level of cortisol (Markowitz *et al*, 1981, Pocock *et al*, 1989). This suggests that the pattern of variation in serum phosphate ions throughout the day may be species specific.

The lack of significant diurnal variation observed for protein in this study is consistent with the role of protein in the maintenance of blood volume, pH and blood pressure, all of which are normally kept within a narrow range as explained previously by Stukie (1976). Studies in man, and mice, have however reported significant diurnal variation in serum total protein over the 24 hour of the day (Klein *et al*, 1985; Berezkin *et al.*, 1988).

The highest concentration of urea recorded in this study was during the afternoon (12.00 – 18.00 hr). In man, mice and the eagle owl, urea concentration is highest during the dark (between 1800 and 2400 hr). This was explained to be due to reduced renal function consequent upon reduced metabolic activity in these species (Berezkin *et al.*, 1988; Pocock *et al*, 1989). The situation in the chicken used in this study, is obviously different from this.

Cholesterol and triglycerides in this study showed significant diurnal variation as was earlier reported in raptor birds (Garcia-Rodriguez, *et al*, 1987).

Variation in creatinine during the day reflects degree of muscular activity. The lack of significant diurnal variation observed in serum creatinine in this study is hence interesting, as the local chicken engages in marked muscular activity throughout the day. In the mice, the report on diurnal variation in serum creatinine is at variance with our observation here, since significant circadian variation in serum creatinine has been demonstrated in this species (Berezkin *et al.*, 1988). Three of the four serum enzymes measured in the present study, exhibited robust diurnal variation, only GOT did not exhibit significant diurnal variation in these chicken.

Result of the present study while corroborating some earlier studies, is at variance with some others. This is not unexpected as it has been shown that several ambient factors like photoperiod, food availability, temperature, geographical location etc, affect the Physiology of birds under

natural condition. Of this lot, light-dark cycle is said to exert most powerful influence on the circadian rhythm in most organisms (Moore. Ede, 1986, Rintamaki *et al*, 1986).

Biorhythms of many haematological characteristics in man and animals, form a very important part of the overall regulation of their physiology and of functions of organs like kidney (creatinine, urea) and liver (enzymes) Berger *et al.*, 1980; Berezkin *et al.*, 1988).

Current effort in our laboratory is directed towards studying biorhythms, using more frequent (hourly, half-hourly) sampling paradigm and different species of animals. This we are sure, will more accurately reveal, the pattern of diurnal variation in physiological parameters in animals in a tropical climate like Nigeria.

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REFERENCES

Aschoff, J., Fatranska, M., Doerr, P., Stamm, D. and Wisser, H. (1971) Human Circadian rhythms in continuous darkness: entrainment by social cues. Sci. 171, 213 – 215.

Bikley, S. (1982) Yearly review circadian organisation in mammals and birds Photochem. Photobiol. 35, 887 – 890.

Berezkin, M.V., Gratsinskii, Y.N., Kudinova, V.F. and Chusovkova, T.S. (1988). Seasonal and circadian rhythms of blood biochemical parameters in mice. Bull. Exptl. Biol. Med. 104, 1641 – 1644.

Berger, J. Circannual rhythms in the blood picture of laboratory rats. Folia Harmatol. Liepzig. 107, 54 – 60 (1980).

Cloudsley-Thompson, J.L. (1980) In: Biological Clocks: Wiedenfeld and Nicholson, London.

Coles, E.H. (1986) Veterinary Clinical Pathology. 4th edn. (Ed. E.H. Coles). W.B. Saunders Company, Philadelphia, U.S.A.

Garcia-Rodriquez, T., Ferrer, M., Recio, F. and Castroviedo, J. (1987) Circadian rhythms of determined blood chemistry values in buzzards and eagle fowl. Comp. Biochem. Physiol. 88A, 6663-669.

King, E.J. and Armstrong, A.R. (1934) A convenient method for determining serum and bile phosphatase activity. Can. Med. Assoc. J. 31, 276 – 281.

Klein, R. Bleiholdes, B., Jung, A. and Erkert, H (1985)**.** Diurnal variation of several blood parameters in the owl monkey, Aotus trivirgatus griseimembra. Folia Primatol. 45, 195 – 203.

Makinde, M.O. and Olowookorun, M.O. (1987) The erythrocyte osmotic test of Nigerian piglets. Bull. Anim. H1th. Prod. Afr. 34, 25 – 28.

Markowitz, M., Rotkin, L., Rosen, J.F. (1981) Circadian rhythms of blood minerals in humans, Sci. 213, 672 – 674.

McPherson, K., Healy, M.J.R., Flynn, F.V., Piper, K.A. J., Garcia-Webb, P. (1978) The effects of age, sex, and other factors on blood chemistry in health. Clin. Chim. Acta. 84, 373 – 379.

Mills, J.N., Minors, D.S. and Waterhouse, J.M. (1978). Exogenous and endogenous influence or rhythms after sudden time shift. Ergonimic, 21, 755 – 761.

Minors, D.S. and Waterhouse, J.M. (1981) Circadian rhythms and the human, Wright, P.G.S., Bristol.

Minors, O.S. and Waterhouse, (1986). J.M. Circadian rhythms and their mechanisms. Experintia. 42, 1 – 108.

Moore-Ede, M.C. (1986) Circadian physiology of the circadian timing system: predictive versus reactive homeostasis. Am. J. Physiol. 250, 735 – 752.

Oyewale, J.O. (1987). Haematological and plasma biochemical values of two breeds of domestic fowls in a tropical environment. Anim. Tech. 38, 49 – 53.

Oyewale, J.O. and Durotoye, L.A. (1988) Osmotic fragility of erythrocytes of two breeds of domestic fowls in the warm humid tropics. Lab. Anim. 22, 250 – 254.

Pocock, S.J., Ashby, D., Shaper, A.G., Walker, M., Walker, Broughton, P.M.G. (1989) Diurnal variations in serum biochemical and haematological measurements. J. Clin. Pathol. 42, 172 – 179.

Reitman and Frankel, (1970) Clinical Laboratory Methods and Diagnosis. In: Textbook on Laboratory Procedures and their Interpretation. 7th edn. Anderson and Roy, University of California.

Rintamaki, H., Hissa, R., Etches, R.J., Scanes, C.G., Balthazar, J. and Saarela, S. (1986) Seasonal changes in some plasma hormones in pigeons: diurnal variation under natural photoperiod with constant or seasonally changing ambient temperature. Biochem. Physiol. 84A, 33 – 38.

Schales, P. and Schales, S.S. (1941) A simple and accurate method of the determination of chlorides in biological fluids. J. Biol. Chem. 140, 879 – 884.

Schalm, O.W., Jain, N.C. and Carroll, E.J. (1975) Veterinary Haematology, 3rd edn. Pp 66 – 78. Philadelphia, Lea and Febiger.

Sturkie, P.D. (1976) In: Avian Physiology. Springer, New York.

Twiest, G. and Smith, C.J. (1970). Circadian rhythms in the blood glucose level of chickens. Comp. Biochem. Physiol. 32, 371 – 375.

Toro, G. and Ackermann, P.G. (1975) In: Practical Clinical Chem. 1st edn. (Eds. G. Toro, and P.G. Ackermann) Little Brown and Company, Inc. Boston, U.S.A.

Touitou, Y., Touitou, C and Bogdan, A. (1986) Differences between young and elderly subjects in seasonal and circadian variations of total plasma protein and blood volume as reflected by haemoglobin, haematocrit and erythrocyte count. Clin. Chem. 32, 801 – 804.

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