DIURNAL VARIATIONS IN TESTOSTERONE, PROLACTIN AND HAEMATOLOGICAL PARAMETERS IN THE NIGERIAN INDIGENOUS COCK

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ABSTRACT
Hormones, such as testosterone and prolactin, play essential roles in growth, reproductive and physiological functions of animals. This research focused on the diurnal variation in the concentrations of testosterone and prolactin in Nigerian indigenous cocks, exploring the impact of photoperiod on hormone secretion. Six (6) adult cocks of domestic chicken each from a group of four (4) birds weighing about 1.00 kg to 1.50 kg were used in this study. Blood samples were collected every two hours over a 24-hour period, with subsequent analysis for testosterone and prolactin concentrations. Blood parameters such as white blood cell count, red blood cell count and hemoglobin concentration were also examined. Results indicated significant ($P<0.05$) diurnal variations in testosterone secretion peaking in the morning (1.27 ng/mL) and declining in the evening (0.79 ng/mL). Prolactin, while not significantly ($P>0.05$) different over the 24-hour period, exhibits diurnal variations. Red and white blood cell counts are higher during daylight hours, emphasizing the influence of activities and exposure to stress during the day. In Conclusion, this research showed that photoperiod have significant effects on the secretion of testosterone and considerable diurnal variations in the concentrations blood parameters examined. This highlights the importance of considering diurnal variations and photoperiod in hormone concentration studies, providing valuable insights into the reproductive and physiological dynamics of Nigerian indigenous cocks.
Keywords: Blood, Cock, Diurnal, Photoperiod, Prolactin, Testosterone

INTRODUCTION

In Nigeria, indigenous chicken holds significant prominence, constituting a substantial 80 % of the poultry types reared (RIM, 1992). These birds exhibit a remarkable ability to endure harsh weather conditions and adapt to challenging environments, thriving on scavenging for a substantial portion of their sustenance and demonstrating a robust immunity to endemic diseases (RIM, 1992). Furthermore, their organoleptic properties, particularly in terms of taste and flavour, surpass those of commercial broilers (Benabdel *et al*., 2001), making them a preferred choice, especially in rural and semi-urban areas. Understanding the physiological factors influencing poultry growth and development is essential. Hormones, as potent chemical substances secreted by specialized glands, play a pivotal role in regulating various aspects of animal physiology and behaviour (Berger, 1983). These compounds, encompassing steroids, proteins, peptides, or amino acid derivatives, exert substantial influence over growth, development, metabolic rate, blood pressure, digestion, behaviour and reproduction (Haus and Touitou, 1994). Investigating the intricate relationship between hormones and poultry physiology is crucial for a comprehensive understanding of their biological processes.

In poultry, the concentration of growth hormones, insulin, glucagon, prolactin, adrenal corticoids, and thyroid hormones in blood plasma exhibits variations related to circadian rhythms, environmental factors, and nutrition (Haus, 1996). Light and temperature, among other environmental parameters, significantly influence the diurnal patterns in prolactin and testosterone concentrations, impacting the growth and sexual development of birds (Lewis and Morris, 2006). Consequently, this study aims to explore the diurnal variation in testosterone and prolactin concentrations in Nigerian indigenous cocks, the differences in daytime and night time hormone serum concentrations and some other blood parameters.

MATERIALS AND METHODS

Experimental site and Animal Management: The research was carried out at the Veterinary Physiology laboratory of the College of Veterinary Medicine, Federal University of Agriculture Abeokuta. Abeokuta is located in Southwest Nigeria (Latitude 7° 3’ 39” N and Longitude 3°20’ 54E).

After procurement the cocks were kept in a communal housing unit, measuring 1.5 x 1.5 x 0.3 m. The birds were treated with broad spectrum antibiotics (Oxytetracycline) and anti-stress (multivitamin) as they acclimatized. Birds were allowed thirty (30) days of adaptation to the new environment and were fed with commercially-prepared adult poultry feed, and water was provided *ad-libitum*.

Blood sampling*:* Blood collection was done 2-hourly for twenty-four (24) hours, beginning from, (08.00h), and ending at 08.00h of the following day. During the period of darkness, blood collection was done under a deep green light of low watt. About one (1) mL of blood was collected through the jugular vein and wing vein where jugular was no longer accessible from each bird, the sample collected was divided into two portions, one aliquot into anticoagulant EDTA bottles which was used for analysis of Red blood cell (RBC) count, Haemoglobin (Hb) concentration, and White blood cell (WBC) count. Another aliquot sample was collected into eppendorff tube and allowed to clot was thereafter centrifuged at 3,000 rpm for 10 minutes at room (28□), to obtain serum for the analysis of testosterone and prolactin.

Hormonal Profile Evaluation: Testosterone and prolactin were assayed respectively with the serum samples using testosterone and prolactin hormone ELISA kits manufactured by INTECO®. 50 µL each of the calibrators (0-5), control reagent and serum samples were dispensed into each of the wells of the microplate. The various concentrations of testosterone in the serum samples were determined by locating the average absorbance on the graph, locating the intersecting point on the curve and reading the concentration from the horizontal axis of the graph. In likewise manner the Prolactin ELISA assay was carried out following the manufacturer's procedure.

Statistical analysis: Data obtained are presented as Mean± standard error of means. The effect of sampling time upon serum hormone concentrations was examined with repeated-measures ANOVA, Student T Test was used to compare between daylight and night time levels for blood parameters using Graphpad prism 4 statistical software, statistical significance was set at $P<0.05$.

RESULTS AND DISCUSSION

The prolactin and testosterone secretion pattern is as presented in Fig 1 and 2, respectively. Prolactin, a hormone associated with physiological processes like osmoregulation and reproduction, displays notable variations throughout the day. At 08:00hr, the mean concentration is 3.68 ng/mL, indicating an initial level in the morning. Subsequently, at 10:00hr, there is a decrease to 3.01 ng/mL, followed by a peak at 12:00hr with a mean concentration of 4.17 ng/mL. This mid-day peak suggests a potential role of environmental and circadian factors influencing prolactin secretion in indigenous chickens. In contrast, testosterone, a key hormone influencing male reproductive functions, also shows distinct fluctuations. At 08:00hr, the mean concentration is 0.73 ng/mL, indicating a baseline level. A significant ($P<0.05$) increase is observed at 10:00hr (1.27 ng/mL), possibly correlating with the reproductive activity associated with the morning hours. The testosterone concentration then fluctuates, reaching a trough at 18:00hr (0.26 ng/ml) and subsequently showing a moderate increase at 22:00hr (0.79 ng/mL). These variations suggest a potential circadian influence on testosterone secretion in the indigenous cocks. Rhythms in prolactin and testosterone concentrations are related to light and temperature among environmental parameters, day length and wavelength of light are very important factors for growth and sexual development in birds (Lewis and Morris, 2006).

In this study it was observed that prolactin and testosterone showed diurnal variations. The concentration of these hormones is time and photoperiod sensitive. This is in agreement with Heiblum (2000) who stated that prolactin and some other hormones like cortisol and testosterone are secreted in a pulsatile rhythm, usually influenced by photoperiod, social stress and other environmental factors. In Prolactin, there was no significant ($P>0.05$) difference in its secretion over the period of twenty-four (24) hours, however, secretion shows diurnal variations in nature. This finding contradicts the report of Benker *et al.* (1990) who reported that there is significant difference in the concentration of Prolactin in the daylight time and night time with peak at night time in birds.

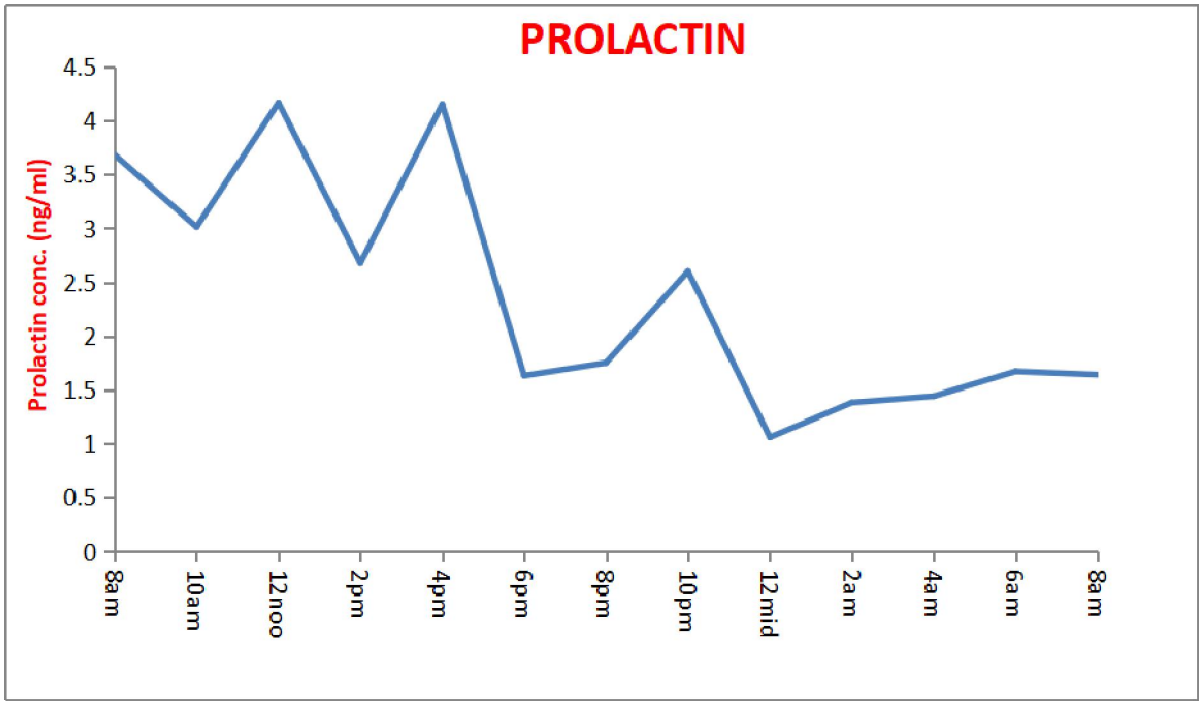


Fig 1: Prolactin secretion pattern within 24 hours

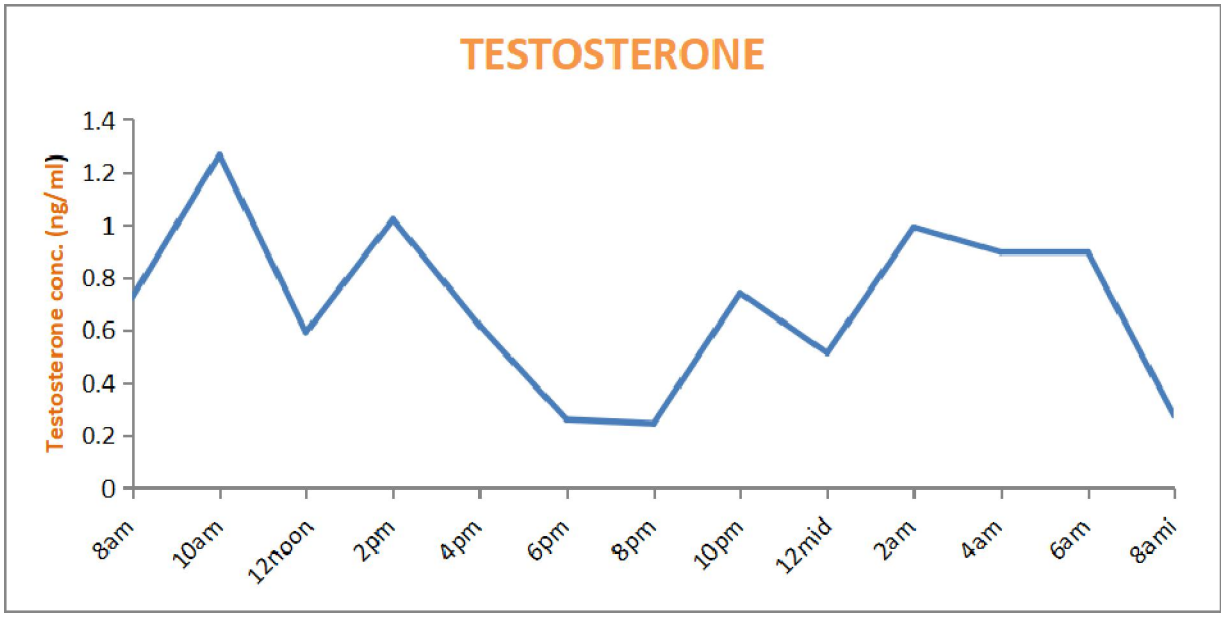
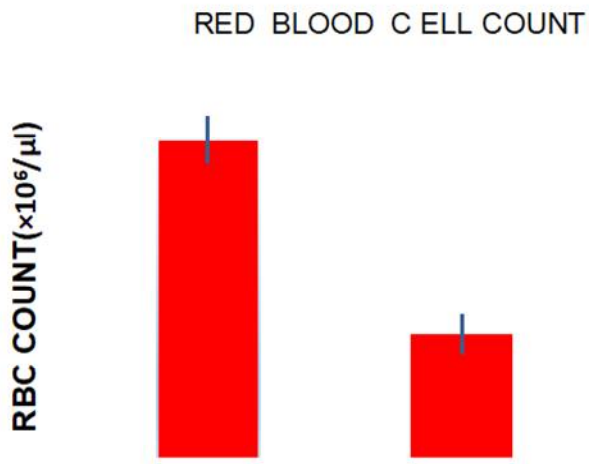


Fig 2: Testosterone secretion pattern within 24 hours The variation in the red blood cell (RBC) count over a 24hr period is as displayed in Fig 3 while the variation in white blood cell (WBC) count over a 24hr period is as displayed in Fig 4. The WBC count, a marker of the immune system's activity, exhibits a substantial decrease from 12:00hr (16330.50±623.32 × 10^9/L) to 6:00hr (1400.00±1003.76 × 10^9/L). This decline may be indicative of a diurnal variation in immune response, with the body potentially being more vigilant against infections during the daytime. However, the considerable standard deviations suggest individual variability and the need for further investigation into potential influencing factors. In contrast, the RBC count observed a general decrease over the measured time points. This could be attributed to factors such as hydration status, circadian rhythm, or physiological adaptations to the time of day. The decrease may also reflect the normal turnover of red blood cells in the body, as they have a finite lifespan.



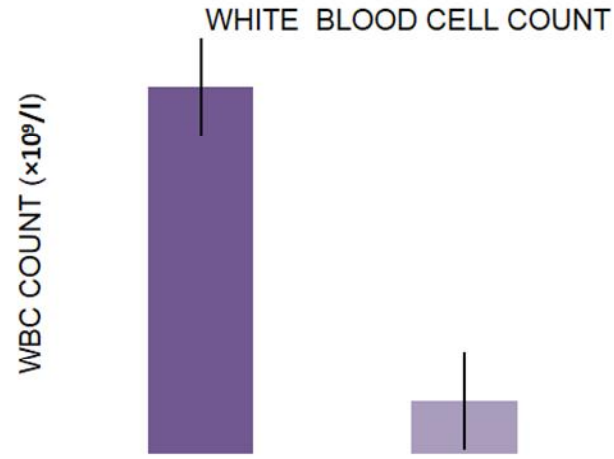


Fig 3: Variation in RBC (daylight to night time) Fig 4: Variation in WBC (daylight to night time)

CONCLUSION

Photoperiod amongst other factors has a significant role to play in the secretion of hormones, thus consideration of time is imperative in concentration determination of hormones. In the study carried out, the serum concentration of testosterone and prolactin were pulsatile in nature and showed considerable effect of diurnal variations in its secretion. Activities associated with daylight remain a primary factor in the high level of blood cell compared to the low levels seen during night time. These findings suggests caution in one time sampling for repro-physiological decisions.

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