PHOTOPERIODIC MODULATION OF CIRCADIAN OSCILLATIONS OF IMMUNE PARAMETERS OF BROILER CHICKS DURING BROODING PHASE: IMPLICATIONS FOR IMMUNE FUNCTION AND HEALTH.

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ABSTRACT
An experiment was conducted to study the effect of photoperiod on the circadian rhythms of immune cells/organs of broiler chicks during brooding. A total of Forty-eight clinically healthy day-old broiler chicks were assigned to two groups; continuous lighting (CL) and restricted lighting (RL). Extended lighting was provided in CL group using LED bulbs at dawn (18.00 h) till dusk. At day-15 of brooding, four broiler chicks from each group were randomly selected, weighed, humanely slaughtered and 3.5mL of blood collected at five-hour intervals over a 24 h period. The bursae of Fabricius and spleens were harvested from the sacrificed broiler chicks and weighed. The data generated were analysed (Student T-Test) and the Single cosinor application®. The results from haematology and lymphoid organs did not show any significant (P > 0.05) differences in both groups. However, the analyzed results from the Single cosinor application® showed the mesors, amplitudes and acrophases in the CL group for heterophil and lymphocyte to be; 26.6 ± 3.50 %, 7.3 %, 09.10 h; 70.0 ± 3.97 %, 6.0 %, 19.7 h and for RL group as 26.0 ± 1.06 %, 0.4 %, 02.30 h; 68.0 ± 3.80 %, 9.7 %, 13.20 h, respectively. It was concluded that CL delayed the acrophases of the heterophils and lymphocytes (early photophase and scotophase), respectively, while the acrophases of heterophil and lymphocyte were restricted to the late scotophase and mid-photophase in the RL group. These findings show that continuous lighting disrupts rhythm of immune cells which may lead to poor immune response and disease resistance in brooded broiler chicks.
Keywords: Broiler chicks, Brooding, Circadian oscillation, Continuous lighting, Immune cells

INTRODUCTION

Vaccination failures and threat of disease outbreaks in flocks has been a serious challenge to poultry farmers (Birhance and Fesseha, 2020). Industry practice during brooding by farmers ensure provision of continuous lighting to encourage adequate feed intake and growth (Arowolo *et al.* 2018; Cobb-Vantress, 2020). Light pollution suppresses immunity by disrupting immune cell rhythms, subsequently affecting immune cell function, which renders the birds more susceptible to infectious agents (Silver *et al.,* 2019; Cheng *et al.* 2020). Makeri *et al.* (2017) and Li *et al.* (2023), in their studies on poultry under natural light – dark cycle, reported the existence of daily oscillations in blood cellular elements, with the rhythms of lymphocyte and heterophils, restricted to the photophase and scotophase, respectively. These cells play important roles in immune surveillance and pathogen defense. Circadian regulation of immune organ development by light influences antibody production (Oso *et al.* 2022; Liang *et al.,* 2023). B lymphocytes which produce antibodies, mature in bursa of Fabricius, and are essential for adaptive immunity (Kaiser, 2020). The bursal index is a valuable indicator of immune status (Glick, 1983). Insights on enhancing the immune response in broilers is crucial to minimizing antimicrobial use. Giving the importance of light in regulating immune functions, there is need for poultry scientists to investigate the effects of light duration on immune cell dynamics during early growth phase under our local conditions. Therefore, a study was carried out to get novel insights on the subject with the aim of improving immune health and minimizing vaccination failures, thereby, enhancing poultry productivity.

MATERIALS AND METHODS

The study was conducted in October, 2024 at the College of Agriculture and Animal Science, Mando-Kaduna (11° 10′N, 07° 38′E) located in the Northern Guinea Savannah zone of Nigeria. Approval for the experimental protocol was obtained from the Institutional Animal Care and Use Committee of Department of Animal Health and Husbandry, College of Agriculture and Animal Science, Mando road, Kaduna.

Forty-eight (48) day-old clinically-healthy Arbor Acres hybrid unsexed broiler chicks with similar weights, were stabilized for three days prior to commencement of experiment. The chicks were kept on deep litter brooding rooms in two partitions (1,200 x 2,000 cm) of 24 birds each, continuous lighting (CL) and restricted lighting (RL), they had access to water and standard diet (Chikun Feeds®, Olam Feeds and Flour Mills, Kaduna, Nigeria) *ad libitum* during the experimental phase. Extended lighting was provided in CL group using LED bulbs at dawn (18.00 h) till dusk (06.00 h). Broiler chicks on RL were in pens fully shielded from extraneous lighting at dawn (18.00 h) till dusk (06.00 h). Adequate brooding temperatures were maintained during study.

Thermo-hygrometric readings were recorded using dry and wet-bulb thermometer (DTH 1; Clarke International Ltd., Essex, UK), placed in the middle of the pen (Silanikove, 2000) just above the head level of the chicks during the brooding period on days 1, 7 and 14. The relative humidity (RH) values were calculated using Osmon's hygrometric table (Narinda Scientific Industries® Haryana, India).

On day 15 of brooding, four birds from each group were humanely sacrificed (Minka and Ayo, 2013) at 5 hour-intervals. 3.5mls of blood was collected into the blood sample tubes containing Ethylene Diamine Tetra Acetic acid (EDTA) and labelled accordingly. Bursectomy and splenectomy were carried out by careful dissection of the abdomen using a scalpel blade to expose the visceral organs. The bursae of Fabricius and spleens were harvested and weighed. The blood samples were preserved on ice packs, before laboratory analyses for hematological parameters.

Data Analysis

The data generated from the study were analysed using the student T-Test. The results were presented as mean ± SEM. Evaluation for rhythmicity was carried out using the Single cosinor procedure (Cosinor Online Software, Molcan, 2019). Three rhythmic parameters were characterized and determined: mesor (M) rhythm adjusted mean level, amplitude (A), acrophase (Φ) time at which peak of rhythm occurred. The mean level of the rhythm was computed as the arithmetic mean of all values in the data set. The amplitude of the value rhythm was calculated as half the maximum – minimum range of the oscillation (which was computed as the difference between the peak and trough). The results are presented as tables and chronograms. Values of $P < 0.05$ were considered significant.

Results, Discussion and Conclusion

The meteorological data of ambient temperature and relative humidity during the period was 29.3 ± 1.2 °C and RH 79.3 ± 3.5%. The recorded values were within range for brooding chicks (Brandt *et al.,* 2022).

Table 1: Overall values for haematological and lymphoid organ parameters

Parameters	CL	RL
Heterophil count (%)	28.18 ± 3.50	26.20 ± 1.06
Lymphocyte count (%)	68.28 ± 3.97	68.26 ± 3.80
Heterophil/ Lymphocyte Ratio (H/L)	0.44 ± 0.09	0.39 ± 0.03
Bursa of Fabricius Index (BBI)	0.24 ± 0.03	0.23 ± 0.03
Spleen weight (g)	0.43 ± 0.01	0.42 ± 0.02
Live weight gain (g)	608.50 ± 15.8	582.30 ± 10.3

H/L – Heterophil/lymphocyte ratio., BBI – Bursa of Fabricius Index. [a, b] = Means with different letters are significantly ($P < 0.05$) different.

The overall values for haematological parameters, lymphoid organs and live weights were similar ($P > 0.05$), however, the heterophil count and live weight gain in chicks on CL were slightly higher compared to those on RL. The overall value in H/L ratio, though not significant, agrees with Kannan *et al.* (2002), who attributed its increase to stress, resulting from corticosteroid production. Besides, there was a significant increase in H/L ratio in broiler chicks subjected to CL during the early photophase (07.00 h) compared to those on RL, this suggests that CL had stressful effect on those chicks during the early photophase, and that the production of melatonin (hormone produced at night) decreased the adverse effect of CL (Reiter *et al.,* 2004). Sahin *et al.* (2004), noted that physiological functions such as, immune response, production of antioxidant enzymes, glutathione peroxidase and catalase are influenced by melatonin. The diurnal changes of heterophil and lymphocytes in both groups as shown in Figure 1 depicts significant differences at 07.00 h and 02.00 h.

Diurnal fluctuation in the bursa of Fabricius index (BBI) and spleen weights in both groups, were not statistically ($P < 0.05$) different except at 07.00h, suggesting that chicks under RL had a better immune status during the morning hour compared to CL (Glick, 1983). Splenic weights at 07.00 h, though lower has been shown to indicate greater immune competence from a review by Kevin and John (2004). They reported that an assumption of a predictable positive association between spleen size and immune competence may be unjustifiable. Besides, CL is a stressor that affects immune response.

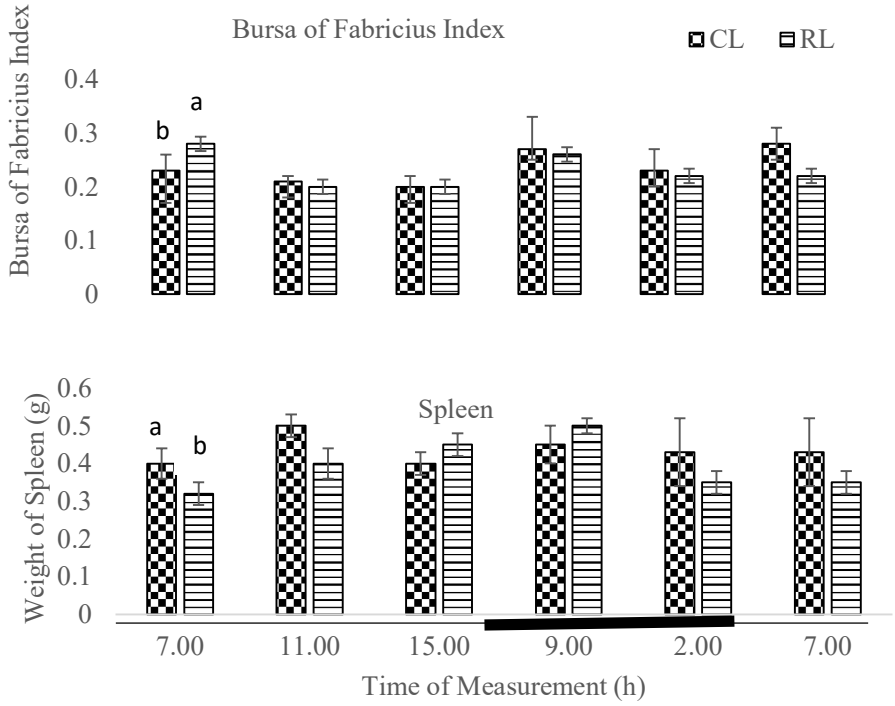

Figure 1: Diurnal variation in bursa of Fabricius index and spleen weights in broiler chicks during the brooding period.

Note: Each data point represents the Mean ± SEM of 4 broiler chicks at each period of measurement. The black horizontal bar denotes the dark phase of the prevailing light/dark cycle. [a, b] = Means with different letters are significantly ($P < 0.05$) different.

Table 2 shows that the mesors of heterophils and lymphocytes were similar, they attained their acrophases at different times of the day. This agrees with the report by Makeri *et al.* (2017), who reported that cellular elements of the blood attain their acrophases at various times of the day based on their zeitgeber. The heterophils had a delayed acrophase at 09.1 h (photophase) in CL group, which differed from the 21.0 h (scotophase) earlier reported by Makeri *et al.* (2017) in broiler chickens under light - dark cycles. The acrophase in the RL group for heterophil was recorded at 02.3 h (scotophase), which agrees with the report by Makeri *et al.* (2017).

Table 2: Circadian characteristics of heterophil and lymphocytes under different photoperiod

Parameters	Mesor (%)	Amplitude (%)	Acrophase (h)
Heterophil			
CL	28.2 ± 3.50	7.3	9.1
RL	26.2 ± 1.06	0.4	2.3
Lymphocyte			
CL	68.3 ± 4.00	6.0	19.7
RL	68.0 ± 3.80	9.7	13.2

The acrophase for lymphocytes in CL group was at 19.7 h, showing it had a delayed acrophase, this disagrees with the result by Makeri *et al.* (2017), but agrees with that at 13.2 h (photophase) for RL (Makeri *et al.,* 2017). The amplitude of heterophils of broiler chicks under CL was higher compared to those under RL. This could be as a result of greater perturbation of heterophil counts experienced by chicks under CL (7.3 %) compared to those under RL (0.4 %).

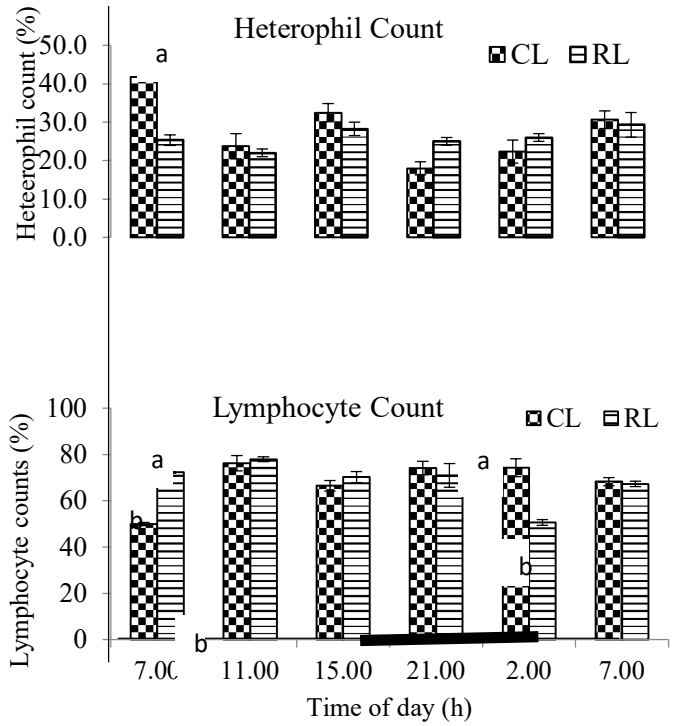

Figure 2. Shows diurnal variation in heterophil and lymphocyte counts in broiler chicks during the brooding period.

Note: Each data point represents the Mean ± SEM of 4 broiler chicks at each period of measurement. The black horizontal bar denotes the dark phase of the prevailing light/dark cycle. [a, b] = Means with different letters are significantly ($P < 0.05$) different.

CONCLUSION

The study concluded that CL delayed the acrophases of the heterophils and lymphocytes (early photophase and scotophase), respectively, while the acrophases of heterophil and lymphocyte were restricted to the late scotophase and mid-photophase in the RL group. It was recommended that farmers should adopt the concept of restricted lighting (RL) regimes which aligns with the broiler chicken's natural circadian rhythm. This will improve immune response and better disease resistance especially with vaccine administration during early morning hour of the day. Restricted lighting also saves feed cost, as the practice of CL does not significantly increase live weights of brooded chicks.

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