TAURINE'S INFLUENCE ON PHOTOPERIODIC ENTRAINMENT OF CIRCADIAN RHYTHMS OF HETEROPHIL, LYMPHOCYTE, AND IMMUNE ORGAN RESPONSES IN BROILER CHICKS DURING BROODING

***Makeri, H. K., Adeiza, A. A., Bassahwa, J. P., Abdulaziz, M. A., Usman, L., Mohammed, A. N., Yusha'u, U., Bolanle, M. A. and Abdulkareem, H. C.**
Department of Animal Health and Husbandry, College of Agriculture and Animal Science,
Division of Agricultural Colleges, Ahmadu Bello University, Mando-Kaduna, Nigeria
Corresponding author: kutamakeri@gmail.com; +234 9067831084; +234 8089288150

ABSTRACT
The effect of taurine on circadian rhythm of immune cells/organs of brooded broiler chicks under continuous lighting was studied. Ninety-six (96) clinically healthy day-old broiler chicks were assigned to groups; continuous lighting (CL), restricted lighting (RL), with plain drinking water, while CL+ T and RL + T groups, had 0.1 % taurine added to their drinking water, all served ad libitum. Light emitting diode (LED) bulbs extended lighting hours at dawn (18.00 h) till dusk. On day-15 of brooding, four broiler chicks from each group on 5 – hourly basis were randomly selected, weighed, humanely slaughtered and 3.5mL of blood collected over a 24 h period. The bursae of Fabricius and spleens were harvested and weighed. The statistics applied to the data obtained were the Student T-Test and the Single cosinor application®. The results did not show any significant (P > 0.05) differences in measured parameters in all groups. However, the Single cosinor application® showed the mesors, amplitudes and acrophases in the CL group for heterophil and lymphocyte to be; (28.2 ± 3.50 %, 7.3 %, 09.10 h); (68.3 ± 4.0 %, 6.0 %, 19.7 h) and for RL group as (26.0 ± 1.06 %, 0.4 %, 02.30 h); (68.3 ± 3.80 %, 9.7 %, 13.20 h), and for CL + T group as (30.3 ± 1.10%. 1.4 %, 4.5 h); (68.7 ± 0.91%, 1.3 %, 23.3 h) and for RL + T group as, (30.3 ± 0.69 %, 1.2 %, 22.0 h); (68.4 ± 3.80 %, 1.3 %, 11.4 h), respectively. It was concluded that continuous lighting disrupted the rhythm of immune cells, however, taurine maintained the rhythmicity of the heterophils only in chicks under RL, but did not modulate that of lymphocytes in brooded broiler chicks under continuous lighting.
Keywords: Broiler chicks, Brooding, Circadian rhythm, Continuous lighting, Immune cells, Taurine

INTRODUCTION
Light-pollution is a probable cause of vaccination failures. Current industry practices of brooding chicks under continuous lighting, could contribute to these failures. Chickens exhibit complex and varied circadian systems (Barrett and Underwood, 1991). These circadian systems have self-regulating clocks located in the pineal gland and hypothalamus of birds (Natesan *et al.,* 2002). Light stimuli as the primary zeigeber in avian species, also decreases melatonin surge at night, which disrupts rhythm responses of physiological activities (Ma *et al.,* 2021). The desire to increase poultry productivity in term of eggs and meat, has caused system modifications and altered practices that ensures continuous production cycles. This has forced farmers to adjust light regimes to the detriment of poultry health (Classen *et al.,* 1991). Various lighting programs have their advantages and challenges. Insufficient or excessive lighting can lead to stress-related behaviours with disruption in circadian rhythms, which negatively impacts welfare of broiler chickens (Olajide *et al.* 2021). Broilers chickens are typically reared under extended photoperiod to allow for increased feed access and to promote growth (Cobb-Vantress, 2020). There is paucity of information on the impact of the practice of continuous lighting during brooding in broiler chicks under our local conditions, hence, the study was carried out.

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.
Ninety-six (96) clinically-healthy day-old Olam hatchery broiler chicks were used for the study. The 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 four partitions (1,200 x 2,000 cm) of 24 birds each. The chicks were assigned to four groups; continuous lighting (CL), restricted lighting (RL), which were served plain drinking water, while CL+ T and RL + T groups, which had 0.1 % taurine added to their drinking water, all served *ad libitum.* Light emitting diode (LED) bulbs extended lighting hours 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. Commercial broiler starter mash (Chikun Feeds®, Olam Feeds and Flour Mills, Kaduna, Nigeria) *ad libitum* was served during the experimental period.

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 post stabilization, four birds from each group were humanely sacrificed (Minka and Ayo, 2016) 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 incision of the abdomen using a scalpel blade to expose the visceral organs. The bursae of Fabricius and spleens were harvested, weighed and recorded. The blood samples were preserved on ice packs, before laboratory analyses for haematology.

Data Analyses

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 AND DISCUSSION

Meteorological measurements during the study showed that the overall dry bulb temperature (DBT) during the study was 29.3 ± 1.2°C and the relative humidity (RH), 79.3 ± 3.5%. %. The recorded values were within established range for brooding chicks (Brandt *et al.,* 2022). Table 1 shows the overall values for haematological parameters, bursar of Fabricius index, spleen and live weight gains of broiler chicks. There were no significant differences in the immune cell counts (heterophil and lymphocyte). Though broiler chicks supplemented with taurine had higher ($P > 0.05$) heterophil counts compared to those that were not supplemented. This finding agrees with what was earlier reported by Sahu *et al.*(2015) and Makeri *et al.*(2017), who noted increases in heterophil counts in broilers administered taurine. Antioxidant studies on taurine shows it possesses cytoprotective effects under stress conditions, and that it acts through various cellular processes (Kim and Cha, 2014; Marcinkiewicz and Kontny 2014).

Table1: Overall values for haematological parameters and immune organs

Parameter	CL + T	RL + T	CL	RL
Heterophil counts (%)	30.3 ± 1.10	30.3 ± 0.60	28.2 ± 3.5	26.2 ± 1.10
Lyphocyte count (%)	68.6 ± 0.90	68.5 ± 0.70	68.3 ± 4.0	68.3 ± 3.20
H/L ratio	0.49 ± 0.05	0.45 ± 0.01	0.44 ± 0.09	0.39 ± 0.03
BB Index	0.23 ± 0.04	0.20 ± 0.04	0.24 ± 0.03	0.23 ± 0.03
Weight of Spleen (g)	0.43 ± 0.01	0.39 ± 0.01	0.43 ± 0.02	0.42 ± 0.02
Live weight gain (g)	520.80 ± 19.9	475.32 ± 15.3	601.1 ± 15.8	582.00 ± 10.3

CL + T – continuous lighting supplemented with taurine, RL + T – restricted lighting supplemented with taurine, CL – continuous lighting, RL – restricted lighting, H/L ratio – heterophil/lymphocyte ratio, BB Index – bursa of Fabricius Index.

Table 2: Rhythm characteristics of heterophil and lymphocyte counts of non-supplemented and taurine-supplemented broilers chicks during brooding

	Mesor (%)	Amplitude (%)	Acrophase (h)
Heterophil			
CL + T	30.0 ± 1.1	1.4	07.45
RL + T	30.5 ± 0.7	1.2	22.00
CL	26.6 ± 3.5	7.3	09.10
RL	26.0 ± 1.1	0.4	02.30
Lymphocyte			
CL + T	68.7 ± 0.9	1.3	23.30
RL + T	68.3 ± 0.7	1.3	11.40
CL	68.3 ± 4.0	6.0	19.70
RL	68.0 ± 3.8	9.7	13.20

CL + T – continuous lighting supplented with taurine, RL + T – restricted lighting supplemented with taurine, CL – continuous lighting, RL – restricted lighting

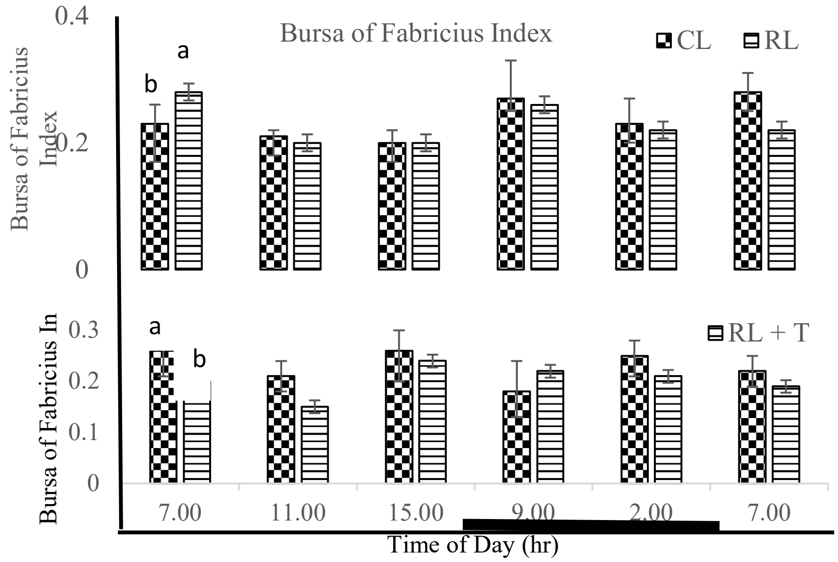

Figure 1. Diurnal variation in bursa of Fabricius index in broiler chicks during the brooding period. Note: Each data point represents the Mean ± SEM of 4 broiler chicks at each period of measurement. The black horizontal bar denotes the dark phase of the prevailing light/dark cycle. [a, b] = Means with different letters are significantly (P < 0.05) different.

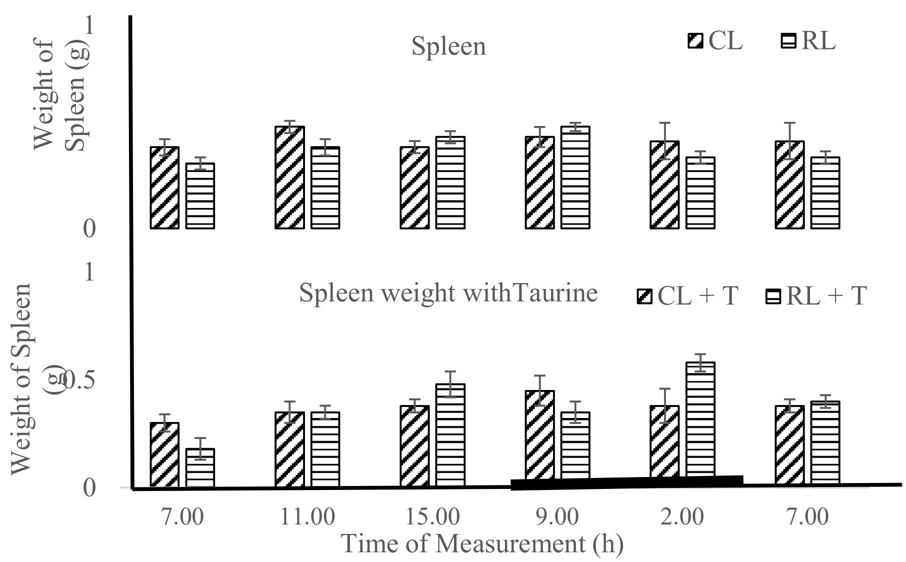

Figure 2. Diurnal variation in spleen weight in broiler chicks during the brooding period. Note: Each data point represents the Mean ± SEM of 4 broiler chicks at each period of measurement. The black horizontal bar denotes the dark phase of the prevailing light/dark cycle. [a, b] = Means with different letters are significantly (P < 0.05) different.

The H/L ratio observed in the current study for broiler chicks supplemented with taurine and those in CL were similar. The overall values in H/L ratio in the various groups 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. Sahin *et al.* (2004), noted that physiological functions such as, immune responses are influenced by melatonin.

Table 2, show that the mesors of heterophils and lymphocytes were similar in all groups, and 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). The acrophase for lymphocytes in CL group was at 19.7 h, showing that 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 %) and taurine's modulatory ability to modulate the amplitude, while advancing the acrophase towards the scotophase as earlier reported by Makeri *et al.* (2017) as compared to those under RL (0.4 %), which were similar to those in the taurine supplemented groups. The observed variation in time of attainment of acrophases may be as a result of taurine's ability to influence circadian clocks that regulate other parameters as observed in the current study. Figure 1, shows diurnal fluctuation in the BBI in all groups. There was a statistically ($P < 0.05$) significant difference in the values at 7.00h, suggesting that chicks in RL group had a better immune status during the morning hour compared to those in CL group (Glick, 1983). However, taurine supplementation reversed the effect of continuous lighting on BBI at 07.00 h till 15.00 h in the CL group.

Diurnal variation in splenic weights in all the four groups (Figure 2), show that broiler chicks under continuous lighting recorded higher diurnal values ($P > 0.05$) compared to those on restricted lighting. In an earlier review, an assumption of positive relationship between splenic size and immunity may be unjustified, and that the inverse of the above is true (Kevin and John, 2004), besides, CL is a stress factor and this may negatively affect immune organs.

CONCLUSIONS

It was concluded that taurine was unable to restore the rhythm of heterophils and lymphocyte counts to the period of scotophase and photophase, respectively, in brooded broiler chicks subjected to continuous lighting. Continuous lighting during brooding did not significantly improved the live weights in broiler chicks. These findings show that continuous lighting disrupts rhythm of immune cells leading to poor immune response, however, supplementation with taurine modulates this pertubation and this may improve immune response to ensure improved disease resistance in brooded broiler chicks under continuous lighting.

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