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Estimation of stress level of broiler chicken reared under different sources of light

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Abstract
A study was conducted to investigate the effect of different light sources *viz*. Incandescent, CFL and LED on the stress level of broiler chicken. One hundred and sixty day old broiler chicks were brooded in battery cages for a week and then distributed randomly into four light treatment group's *viz.*, Natural light (T_1) as Control, Incandescent (T_2), CFL (T_3) and LED (T4) groups having 40 chicks in each which were again subdivided into four replicates of 10 chicks each. The stress levels were estimated by measuring the plasma corticosterone levels and heterophil lymphocyte ratio (H:L) in the blood at second, third and fifth week. The plasma corticosterone level was found to be highest during 2nd week in birds reared under INC light ($11.07^{c} \pm 0.39$) and lowest in 5th week under LED light ($5.17^{a} \pm 0.22$).The results showed that heterophil: lymphocyte (H:L) ratio and levels of plasma corticosterone were significantly ($P \leq 0.05$) higher in the INC group and lowest in the LED group.

Keywords: Stress, light sources, broiler chicken

Introduction
Light plays major role poultry production by creating microclimatic situations that stimulates growth, development and physiological functioning. The lighting programmes within a production system could potentially influence the activity and physiological response like stress of the broilers being raised in that situation [1]. Light stimulation has effects on brain organization that influence behavioral responses, including fearfulness [2]. Light manipulation has been an effective measure to improve poultry production. The light source may affect the effectiveness of housing conditions due to changes in the ambient conditions. Several types of lighting systems, such as incandescent, fluorescent, compact fluorescent, fluorescent tube lighting and high intensity discharge lighting have all been used in commercial poultry housing. Recently, light emitting diode (LED) lamps have been of growing interest in poultry operations because of their high energy efficiency [3], long operating life, availability in different wavelengths [4], low electricity consumption and low rearing cost [5].

The poultry industry has been using the incandescent light bulb since decades to provide illumination in poultry houses for rearing of birds. The 95% of the energy is converted to heat energy by Incandescent lamps, only about 5% is converting energy to light efficiently. Nowadays many energy efficient lights like Fluorescent lights, especially the newer compact fluorescent lights (CFLs) which consumes significantly lower level of power for a similar light output and are presently preferred by the industry for rearing of birds [6]. Similarly, light emitting diodes (LEDs), another energy efficient light with much durability compared to the other types of bulbs and provide a different with better spectrum output that has been found good and more realistic as reported by various workers [7]. By selecting the optimum light source as per the needs of the birds and also taking advantage of the unique spectral requirements for better poultry production, it can be make possible to increase the growth and feed efficiency and reducing unnecessary stress and providing welfare to the birds.

Stress response in poultry may be measured by a variety of methods depending on the degree and duration of the stress being applied. Two such methods include testing of blood samples for relative levels of plasma corticosterone, a short-lived adrenal hormone associated with a "fight or flight" response in the bird, and comparing ratios of white bloods cells, specifically heterophils to lymphocytes (H:L), in the blood. Corticosterone levels have been shown to dramatically increase as a direct response to acute (i.e. seconds to minutes of exposure) stress with levels returning to pre-stress ranges shortly after the stressor has been removed.

In contrast, leukocyte response is slow, taking hours to days to respond to an applied stressor and is, thus, a better indicator of chronic stress [8, 9, 10, 11]. A ratio of peripheral blood circulating heterophils to circulating lymphocytes has been shown to be a method of chronic stress evaluation [12]. Therefore, the present study was undertaken to see the stress level of the broiler chicken reared under different sources of light.

Materials and Methods

To check the impact of various lighting sources on the stress and economics of broilers, the present study was conducted at Student's Poultry Instructional Farm, Faculty of Veterinary and Animal Sciences, SKUAST Kashmir, Jammu and Kashmir, during the months of March-April, under deep litter system management. One hundred and sixty day old broiler chicks were obtained from a commercial hatchery and brooded in battery cages for a week. After one week the chicks were weighed by using an electronic balance and the chicks having similar body weights were distributed randomly into four light treatment groups *viz*, Natural light (T_1) as control, Incandescent (T_2), Fluorescent (T_3) and LED (T_4) having 40 chicks in each which were again subdivided into four replicates of 10 chicks each. Each treatment group was housed in a light proof enclosure. Continuous lighting was provided to the birds similar intensity in all treatment groups was maintained. The light intensity was kept similar in all the treatment groups and monitored regularly using a digital lux meter. The experimental barn was cleaned thoroughly and kept under similar housing and management conditions like floor space, temperature, ventilation, humidity, *ad.lib.* feed and fresh water except sources of light.

Stress was measured through the estimation of heterophil: lymphocyte (H:L) ratio and the level of plasma corticosterone at 2nd, 3rd and 5th weeks of age. Blood was collected from the wing vein of eight (8) birds per treatment into heparinized vials. One drop of blood sample from each bird was immediately transferred to slides and prepared as a smear. The smear was air dried and fixed in methanol. The slides were then stained using Wright Giemsa stain and H:L ratio was obtained following methods outlined by Gross and Siegel [12]. A combination of heterophils and lymphocytes were tallied to the first hundred cells encountered and the ratio was calculated by dividing the number of observed heterophils by the number of observed lymphocytes.

Corticosterone level was estimated by ELISA kit (Calbiotech). The blood samples at 2nd, 3rd and 5th weeks of age from eight (8) birds per treatment was collected and centrifuged to obtain the plasma. The plasma was stored at -20 ^{0}C and all the samples were collectively analysed for the corticosterone levels by standard ELISA method.

Statistical Analysis

The data so obtained was statistically analyzed by analysis of variance (ANOVA) technique as per Snedecor and Cochran [13]. The differences in means of the treatments were compared by Duncan's Multiple Range Test [14].

Results and Discussion

The corticostretone levels (ng/ml) and the Heterophil: lymphocyte ratio (H:L) of broilers at 2nd, 3rd and 5th week of age is shown in table 1 and table 2 respectively. Significant differences were found in the plasma corticosterone levels and H:L ratio of the birds reared under incandescent lighting group (T_2) compared to those reared under CFL, LED and control groups. The corticosterone levels at 2nd week of age was found to be significantly ($P \leq 0.05$) higher in T_2 (11.07±0.39) followed in decreasing order by T_3 (9.19±0.13), T_4 (8.79±0.18) and least in T_1 (8.14±0.10) groups. During 3rd week of age again significantly ($P \leq 0.05$) higher corticosterone levels of 9.54±0.39 were found in T_2 followed by levels of 8.94±0.10, 8.48±0.21 and 8.03±0.12 in T_3, T_4 and T_1 groups respectively. The corticosterone levels at 5th week of age were again found to be significantly ($P \leq 0.05$) higher in T_2 (7.66±0.10) followed by T_1 (6.37±0.14), T_3 (5.96±0.23) and least in T_4 (5.17±0.22) groups. The corticosterone levels were overall found to be lower during the 5th week of experiment for all the treatment groups.

The H:L ratio at 2nd week of age was significantly ($P \leq 0.05$) higher in T_2 (0.55 ± 0.004) followed by T_3 (0.46 ± 0.002), T_4 (0.43 ± 0.002) and T_1 (0.42 ± 0.004) groups. However, no significant difference was found between T_1 and T_4 groups. Same trend was continued in the 3rd week of age with significantly ($P \leq 0.05$) higher H: L ratio was found in T_2 (0.46 ± 0.004) followed by T_3 (0.43 ± 0.007), T_4 (0.42 ± 0.002) and least in T_1 (0.40 ± 0.006) groups. At 5th week age the H: L ratio was recorded as 0.40 ± 0.008, 0.45± 0.004, 0.39 ± 0.004 and 0.38 ± 0.006 in T_1, T_2, T_3 and T_4 groups, respectively. Significantly ($P \leq 0.05$) higher H:L ratio was found in T_2 in comparison to other treatment groups. However, no significant differences were observed among T_1, T_3 and T_4 groups.

Significantly ($P \leq 0.05$) higher levels of plasma corticosterone and H:L ratio found in the birds reared under incandescent lighting group (T_2) than those reared under CFL, LED and control groups might be due to high radiation from the incandescent lamp which caused stress in birds. Stress was also observed in the behavioral activities of the birds reared under incandescent group evidenced by lower levels of comfort and higher levels of aggression. The birds under control group and Incandescent bulb showed significantly ($P \leq 0.05$) higher corticosterone levels which are indicative of stress. The plasma Corticosterone level s was known to be elevated during stress in broiler birds as reported by [15, 16]. This is in good agreement with the findings of Hajra *et al.* [17] who also recorded slightly lower levels of plasma corticosterone in birds reared under fluorescent light than those under incandescent light. Archer [18] also found higher levels of plasma corticosterone in birds reared under incandescent light than the birds reared under LED light. However, in contradictory to the findings of present study, Rogers *et al.* [19] reported significantly higher heterophil: lymphocyte ratio in birds reared under CCFL lamps than the birds reared under incandescent and LED lamps. Olenrawaju *et al.* [20] did not observe any significant difference in the plasma corticosterone levels of birds raised under four different light sources viz. incandescent, compact fluorescent, Light Emitting Diode and poultry specific filtered LED.

Table 1: Corticosterone levels (ng/ml) at different weeks of age of broiler chicks reared under different sources of light.

Age (Weeks)	Treatment Groups			
	T_1(Control)	T_2(INC)	T_3(CFL)	T_4(LED)
2nd week	8.14^{a} ±0.10	11.07^{c} ±0.39	9.19^{b} ±0.13	8.79^{ab} ±0.18
3rd week	8.03^{a} ±0.12	9.54^{c} ±0.39	8.94^{bc} ±0.10	8.48^{ab} ±0.21
5th week	6.37^{b} ±0.14	7.66^{c} ±0.10	5.96^{b} ±0.23	5.17^{a} ±0.22

Means across rows bearing different superscripts differ significantly ($P \leq 0.05$)

Table 2: Heterophil lymphocyte ratio (H:L) of broiler birds at different weeks reared under different sources of light

Age (Weeks)	Treatment Groups			
	T_1(Control)	T_2 (INC)	T_3 (CFL)	T_4(LED)
2nd week	0.42^{a} ±0.004	0.55^{c} ±0.004	0.46^{b} ±0.002	0.43^{a} ±0.002
3rd week	0.40^{a} ±0.006	0.46^{c} ±0.004	0.43^{b} ±0.007	0.42^{b} ±0.002
5th week	0.40^{a} ±0.008	0.45^{b} ±0.004	0.39^{a} ±0.004	0.38^{a} ±0.006

Means across rows bearing different superscripts differ significantly ($P \leq 0.05$)

Conclusion

From the present study it can be concluded that compared to incandescent light, CFL and LED light sources result in lower levels of plasma corticosterone and H:L ratio both of which are indicators of stress. Hence incandescent light sources can be replaced with modern energy efficient light sources (LED and CFL) for better welfare of broilers. However, further detailed studies are needed in this regard to draw a clear conclusion**.**

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