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Pulsatile secretion of luteinizing hormone and GnRH and its relation to pause days and egg production in hens exposed to different wavelengths of light

I J REDDY[1], C G DAVID[2], G RAVI KIRAN[3] and S MONDAL[4]

National Institute of Animal Nutrition and Physiology, Bengaluru Karnataka 560 030 India

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ABSTRACT

The study aimed to establish the effects of red spectrum of light (650nm; treated n, 12) and normal spectrum of light (450nm control, n=12) on GnRH concentration, amplitude and frequency of luteinizing hormone (LH), estradiol (E2ß), progesterone (P4), intersequence pause days and egg production from 62 to 72 weeks old laying White Leghorn hens. Weekly interval profiles of plasma GnRH, LH, E2ß and P4 concentrations were increased in birds exposed to red spectrum of light. At 67th weeks of age blood samples from both the groups were collected at every 3 h for 36 h to study the pulsatile secretion of LH surges. Plasma LH concentration was higher in treated birds with more number of frequencies and amplitude of LH surges in plasma of treated birds. The LH frequencies were more pronounced and advanced during 36 h of sampling at 3 h interval in treated birds. Weekly interval of plasma LH, E2ß and P4 concentrations increased in treated birds from 62 to 72 weeks of age. GnRH concentration was significantly higher in birds exposed to red spectrum of light compared to controls. It is hypothesized that exposure of birds to red spectrum of light caused enhanced GnRH along with LH, E2ß and P4 hormones required for egg formation and egg lay. During 77 days (62–72 weeks of age) of experimental period, egg production was enhanced with lower incidence of pause days even during the later stages of productive period in treated group. In conclusion, higher levels of GnRH, LH, and E2ß and P4 concentration with lower incidence of pause days enabled the birds to lay more eggs even later in the productive period by modulating the wavelengths of light under normal husbandry conditions.

Key words: Egg production, GnRH, Hen, LH surges,Wave lengths of light

The influence of photoperiod on reproductive activity in the male chicken was mediated by light from the red end of the spectrum (Harrison *et al.* 1970). Longer wavelengths are transmitted through neural tissue more readily than shorter wavelengths (Hartwig and Van Veen 1979). This indicates that the interaction between the wavelengths and the quantum energy could be very important for the photo-neuro-endocrine events in the hypothalamus, an area of brain or in both. Therefore, it is particularly important to consider quantum energy (number of photons), while evaluating the effect of different light sources. Conducting the experiment during later stages of the laying period, where intersequence pause days increases as age advances, and therefore, using red spectrum of light during later stages of egg lay (62–72 weeks of age period) may reveal the exact physiological mechanism and factors that regulate the pause days originating from use of different wavelengths of light. Further, results from this experiment will help to determine the optimum lighting regimen to be used in an industry setting, and will help reduce the energy cost associated with incandescent lighting. The purpose of this study was to determine the effect of light wavelength, on GnRH, LH surges, steroid hormones, egg production and intersequence pause days using 2 different wavelengths of light sources during the later stages of production in domestic hen.

Present address: [1-4]Senior Scientists (e mails: ijreddy123 @rediffmail.com; corbon@rediffmail.com; rkgorti@rediffmail.com; sukanto781@gmail.com).

MATERIALS AND METHODS

White leghorn strain of commercial layer birds were maintained on standard layer ration (16% CP and 11.72 MJ ME kg-1) as per the Ranjhan (1993) with a photoperiod of 16hL: 8hD. Birds (24) selected at random and divided into 2 equal groups to study the egg production, GnRH and pulsatile LH secretions, its relation to exposure to different wavelengths of light. The pattern of egg production studied in these birds from 62 to 72 weeks for 11-week period. Beginning at 62nd week the birds in one group were exposed to red spectrum of light of 650 nm wavelength and the other group was exposed to normal white incandescent light of

450 nm wavelength (control group) throughout the experimental period. The intensity of light was uniform within the group without any variation of 0.1 W/m^2 at the bird-head level. Daily egg production was recorded for each hen at the same time for 77 days. Total numbers of pause days and average egg production were calculated from egg production record for the 11-week period (77 days).

Collection of blood samples: Weekly blood samples were collected from 62 to 72 weeks of age. The birds were bled for 36 h at 3 h intervals at 67th week of age 3 h blood samples were collected for 36 h starting at 0600 h to study the pattern of GnRH and LH frequency and amplitude. The sampling took about 1 h and blood samples were collected in the same order to ensure a period of 3 h between each sample. During sampling lights were off (between 10PM to 6AM), hand torches were used during sampling to minimize disruption of the lighting schedule. Portal blood samples were collected according to Sharp *et al.* (1990). Plasma was separated and stored at - 200° C for hormone assays.

Analysis of hormones: LH was estimtated using RIA as described by Sharp *et al.* (1987). The intra and inter coefficient variation for Chicken luteinizing harmones (cLH) was 5.48%and 9.22% respectively with sensitivity of the hormone 0.01 ng/ml per tube. Plasma progesterone and E2ß were estimated using RIA following the Hall and Sufi (1981). Intra and inter assay coefficient of variation for E2ß were 5.11% and 6.98%, respectively and 5.3% and 8.14%, respectively for P4 and the sensitivity of the method was 2 pg/ ml for E_2ß and 25 pg/ml for progesterone. GnRH concentrations were measured in duplicate by RIA as per (Prevot *et al.* 1998). The sensitivity for GnRH was 0.5 pg/ tube, and the intra-assay variability was 3.4%.

Statistical analysis: Measurements were given as mean±SE. The significance of differences between means was analyzed by F test. The data on egg production, LH, E_2ß and P4 were subjected to correlation coefficient analysis to study the influence of the hormones on egg production. Differences were considered significant at a value of P<0.01. The statistical analyses were carried out following the standard method (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

GnRH and LH secretion: There were differences in GnRH concentration between treated and control hens (Fig.2). GnRH concentration was significantly (P<0.01) higher in birds exposed to red spectrum of light over the control birds. GnRH concentration varied from 5.13 to 7.39pg/ml in treated birds whereas it varied from 5.14 0.59 to 6.19 0.66 pg/ml in controls. This increase in LH and GnRH levels is attributed to the exposure to red spectrum of light during the experimental period of 11 weeks.

Plasma LH concentration in control group varied between 2.44 0.18 ng/ml and 3.53 0.6 ng/ml and 2.81 0.22 ng /ml and 5.12 0.14 ng /ml in the treatment group during 62 to 72 week of age (Fig. 2).

Three hourly secretion of preovulatory LH surges in treated birds occurred mostly between the 9AM to 12 noon with a highest concentration of LH 6.41 0.29 ng/ml, whereas these surges in controls occurred around 2AM to 4PM with highest concentration of 5.31 0.12 ng/ml (Fig. 3). Both LH and GnRH followed a similar pattern of secretion.

Steroid hormones: The plasma E_2ß level in control group varied between 221.95 1.49 pg/ml and 249.99 0.69 pg/ml and from 249.85 1.59 pg/ml to 290.65 1.99 pg/ml in the treatment group during 62 to 72 weeks of age (Fig. 1). Progesterone concentration in the 2 groups also followed a similar pattern as estradiol (Fig.1). However, intermittent hormonal fluctuations were observed in both the groups. In Birds exposed to red spectrum of light, egg production was positively correlated with E_2ß (r=0.61), P4 (r =0.75), LH (r =0.62), GnRH (0.77) and pause days were negatively correlated with E_2ß (r = -0.83), P4 (r = -0.69) and LH (r =- 0.64) and GnRH (r = -0.65).

Egg production parameters: Mean weekly egg production

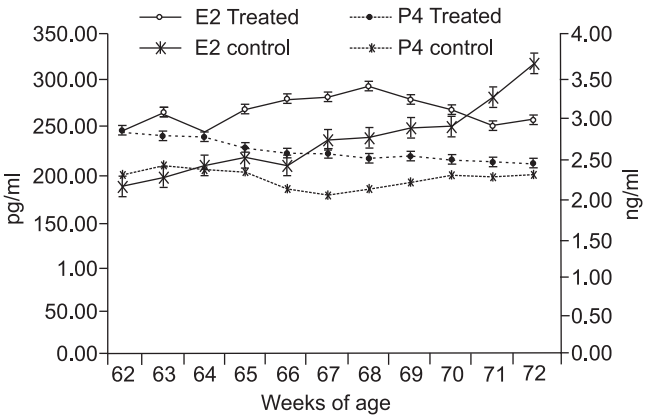

Fig 1. Mean±SE. Plasma estradiol-17 beta (pg/ml) and progesterone (ng/ml) concentration in birds exposed to red spectrum of light (650nm) and normal spectnum of light (450 nm)

in birds exposed to red spectrum of light from 62 to 72 weeks of age was significantly (P<0.01) higher than the controls (Table 1). It fluctuated between 3.71±0.64 and 5.59±0.19 and 3.52±0.36 to 4.31±0.29 in birds of treatment and control groups respectively. Mean weekly egg laying gradually increased in birds exposed to red spectrum of light and decreased in controls. The differences in egg production per week per bird was significant between 2 groups from 64 weeks of age to 72 weeks of age. The % of pause days during the 11 week period (77 days) (Fig.4) were 28.07 0.21 in the treatment group as against 47.93 0.22 days in control group. Egg production in treatment group between 62 to 72 weeks of age, improved by 19.83% over control group (Fig.4).

These results demonstrated for the first time the effect of light quality on the reproductive traits of domestic laying

Table 1. Weekly (mean ± SEM) egg production and % of egg production between control and treated birds

Age in weeks	Mean±S E weekly egg production		% of egg production	
	Control	Treated	Control	Treated
62	4.42±0.33	3.71 ±0.64	63.26	52.99
63	4.31±0.29	3.85 ±0.23	69.57	54.99
64	3.52±0.36	4.71*±0.29	50.28	67.28*
65	4.42±0.16	5.00*±0.26	63.14	71.42*
66	4.28±0.19	5.14*±0.28	61.22	73.42*
67	4.14±0.26	5.01*±0.15	52.99	71.57*
68	3.71±0.26	5.14*±0.21	52.99	73.42*
99	3.85±0.33	5.52*±0.28	54.99	78.85*
70	3.52±0.24	5.59*±0.22	50.28	79.85*
71	3.85±0.25	5.58*±0.38	54.99	79.71*
72	4.14±0.43	5.59*±0.19	59.14	79.85*

Control and treated, n=12; *, differ significantly at (P<0.01) between the groups. % of egg production differ significantly (P<0.01) between control and treated group after 2 weeks.

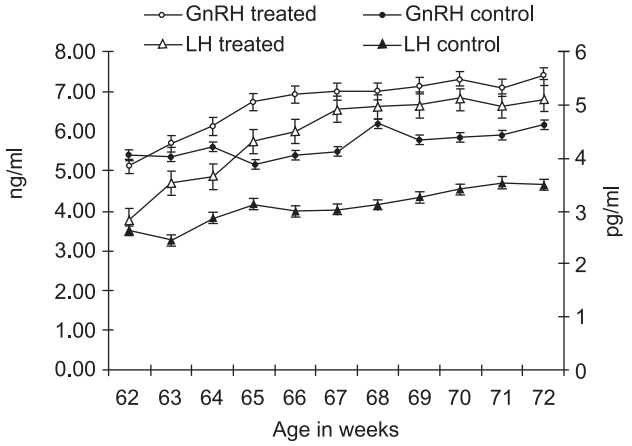

Fig 2. Mean±SE. plasma GnRH (pg/ml) and LH (ng/ml) in birds exposed to red spectrum of light (650nm) and normal spectrum of light (450 nm).

hen during the later stages of egg lay. The finding that there were differences in GnRH levels between treated and control hens suggested that changes in the synthesis of GnRH are the immediate cause of increased LH secretion in treated hens. These changes are attributed to the fact that photons of longer wavelengths (red spectrum of light, 650nm) penetrate the hypothalamus more effectively than photons of short wavelengths (normal incandescent light, 450nm) (Hartwig and Van Veen 1979). This is further supported by the findings observed in turkeys, suggesting that wave lengths from the red spectrum have the ability to penetrate the skull and directly stimulate the hypothalamic photoreceptors, while wavelengths from the blue-green spectrum barely penetrate the brain and mainly stimulate the retina (Shimizu *et al.* 2006, Joseph *et al.* 2009). Though effective penetration through the skull, red spectrum of light directly acts on hypothalamic

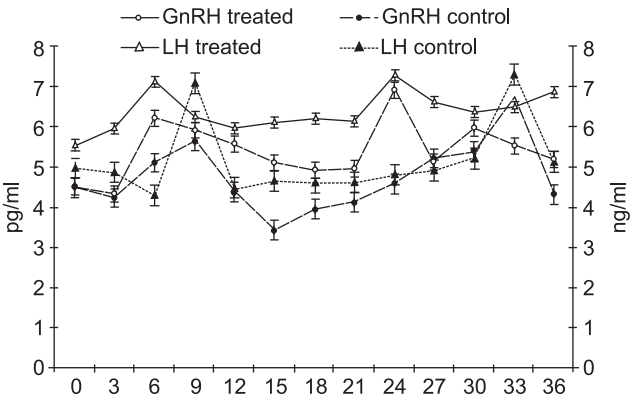

Fig 3. Three hourly mean±S E plasma GnRH 9pg/ml) and LH (ng/ml) concentration in treated (650 nm) and control (450 nm) birds.

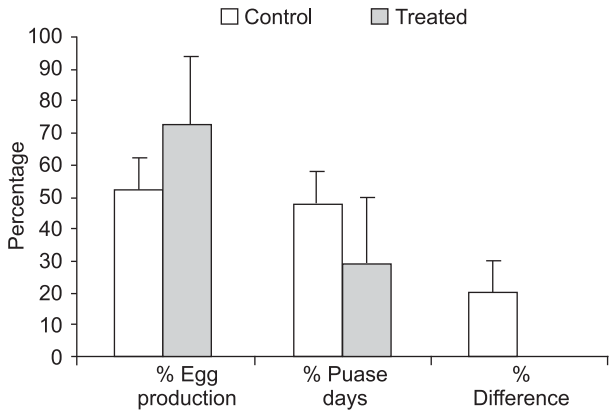

Fig 4. Difference in percentage of egg production % pause days and percentage of difference in egg production between control and treated birds.

extra-retinal photoreceptors to stimulate reproductive function (Menaker and Underwood 1972) thus account for increased GnRH concentration. The decrease in GnRH levels found in control hens is attributed to the less efficient penetrating power of short wavelength of incandescent light thus confirming an earlier finding that hypothalamic GnRH peptide content decrease with decreased egg laying in ageing broilers (Sharp *et al.* 1990). However, a decrease in GnRH found in control hens implied that changes in GnRH release are responsible for decreased gonadotrophin secretion.

The observation that plasma LH frequency occurs earlier in birds exposed to red spectrum of light is similar to the finding that in mammals, a fast GnRH pulse frequency preferentially stimulates LH release whereas a slow GnRH pulse frequency stimulates FSH release (Dalkin *et al.* 1989, Kaiser *et al.* 1997). The decreased LH frequency and concentration observed in the control hens with advancing age, could be similar to those reported in post menopausal women with reduced GnRH pulse frequency (Rossmanith *et al.* 1991) elevated serum FSH levels (Rubin 2000) and

decreased LH levels (Matt *et al.* 1998). The low LH levels may be a consequence of low circulating concentrations of plasma estrogen, compared to treated hens, which exert a reduced stimulatory effect on LH secretion (Dunn *et al.* 2003) in hens than in mammals. Exposure of birds to red spectrum of wavelength, the reproductive system of domestic hen changed from low functional state to a high functional state. High concentrations of E_2ß and P4 were observed within 4 weeks of exposure red spectrum of light. GnRH could attribute increasing levels of steroid hormones in birds exposed to red light to activation of the hypothalamo-pituitary- gonadal axis. Increased GnRH concentration increases E_2ß and P4 levels through elevation of gonadotrophic hormones by inhibiting 20α-hydroxysteroid dehydrogenase enzymes which catabolizes P4 to 20α-dihydroprogesterone (Veldhuis *et al.* 1981) and (Wong *et al.* 1979) and E_2ß levels by stimulating precursors, enzymes and receptors required for E_2ß synthesis at ovarian level. The possible effects of the concentration of GnRH and LH on egg production remains unknown, but high concentration of LH has been shown previously to be associated with initiation of egg production (Bacon and Long 1995) and (Liu *et al.* 2002). Our results indicated that, LH and steroid levels were significantly increased during the initiation of egg production.

Egg production improved in birds exposed to red spectrum of light by 19.83% compared to birds exposed to incandescent light. This increase in egg production is due to the decrease in inter-sequence pause days and increased concentration of GnRH, LH, estradiol and progesterone in these birds. Longer intervals and more pause days suggested that the synchronization between follicular development and LH peaks during the ovulation cycle (and thus the responsiveness of the F1 follicle to LH) is not 'optimal' with ageing but can be improved by stimulating the GnRH through longer wavelengths of light. Secondly, the increased egg production in these birds is related to the pattern of GnRH and LH surges observed in birds exposed to red spectrum of light. GnRH secreted in pulsatile pattern with number frequencies in treated birds over the controls. GnRH surges of higher magnitude occurred at 6 and 24 h in birds exposed to red light in treated birds. In birds exposed to normal spectrum of light, the GnRH frequencies are with lesser magnitude and surges occurred at 33 h. Therefore, egg production is directly related to the occurrence of LH surge frequencies within 24 h but not exceeding 27 h to oviposition. Similarly, the decreased egg production in birds exposed to incandescent light is attributed to occurrence of LH surge frequencies beyond 33 h as observed in this study. Further, longer interval of LH surges of >30 h are associated with more % of intersequence (47.93 0.22) pause days in these birds compared to those hens exposed to red spectrum of light (28.07 0.21). Longer intervals between preovulatory surges of LH and GnRH secretion are associated with decline in egg production late in the reproductive period. Thus, increasing the secretion of GnRH through red spectrum of light increases egg production by decreasing the inter sequence pause days in birds.

To conclude, our results indicated the importance of red spectrum of light (650nm of wavelength of light) on elevated levels of preovulatory LH surges, its duration, steroid hormones, incidence of intersequence pause days and egg production in domestic hen even later in the reproductive period. Increasing the GnRH concentration in birds through stimulatory longer wavelengths of light (650nm) irrespective of the laying period could ameliorate other managemental problems. In present study, understanding of pause days and the involvement of red spectrum of light and GnRH provided the basis for the development of new managemental tools to optimize the egg production in birds.

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