The role of light in biological activities associated with avian growth and reproduction is very well known. Light quality can be defined by three criteria: 1) daily pattern of light-dark exposure; photoperiod, 2) light intensity (brightness), and 3) spectral composition (Andrews and Zimmerman, 1990).

Photoperiod: in birds from subtropical and temperate latitudes, the gradual or abrupt increase in day length (long photoperiod; LP) initiates gonad recrudescence and egg laying. Conversely, reduction in day length (short photoperiod; SP) delays the onset of sexual maturity and may even terminate egg laying activity in birds (Benoit, 1964 and Woodard et al., 1969).

Light intensity: plays an important role in rearing birds, mainly because birds need a certain light intensity in order to be photostimulated (North et al.,1990). Previous study conducted in our lab found a close relationship between light intensity and light spectra of laying hens. We found that by using optimal light spectra, light intensity can be decreased sharply, resulting in a significant decrease in the feed intake of the birds. This method of lighting laying hens can be highly beneficial for farmers, as savings in rearing costs were observed. Furthermore; we found that rearing birds under 0.01W/m^2 at bird head level, significantly reduced feed intake, regardless of light spectra (Rozenboim, et al., 1998).

The role of light in avian reproduction

Light spectra: The chicken eye, in similarity to the human eye, is capable of seeing in a narrow part of the light spectrum (380 to 760nm). Apart from the eyes, birds are equipped with active extra-retinal photoreceptors (ERPRs), located in several parts of the brain, and are involved in transduction of photostimulation. Photostimulation, as affected by different wavelengths, has been discussed previously regarding chickens (Harrison et al., 1970), turkeys (Scott and Payne, 1937), sparrows (Ringoen, 1942), ducks (Benoit, 1964), and quails (Phogat et al., 1985). In general, red light stimulates egg production efficiently, whereas green or blue light have little or no effect. In commercial layers, during the first and second laying season, total egg production was significantly influenced by light color, with the largest amount of eggs produced in the red light-treated group. Furthermore, eggs laid under blue or green light were consistently larger than those laid under the red light (Pyrzak et al., 1987).

Photoreception affects sexual activities: Many avian species are photoperiodic and respond to long photoperiods by activation of the reproductive axis. The neuroendocrine response to photostimulation is reflected by a significant release of gonadotropin-releasing hormone I (GnRH-I)
photoperiodic responses (Menaker et al., 1978; Opel and Proudman, 1988). The VIP within the opsins system has the potential to regulate reproductive function via synaptic interactions all along the trajectory of its axons through the lateral septum (LS) and hypothalamus.

Three studies were conducted in order to investigate the relationship between retinal and extra retinal photoreception in reproductive activities of domestic birds. The first was conducted in by Prof. M.E El Halawani and colleagues on turkey hens, and the second was conducted in our laboratory, on broiler breeder hens (Mobarkey et al., 2010). The objective of both studies was to provide further understanding of the interplay between light spectra photoreception and its association to the modulation of the hypothalamic-pituitary-gonadal axis in both turkeys and broiler breeder hens.

Three light treatments given in the experiments were white light (control), and 2 parallel light circuits, installed in each of the two light-controlled rooms in the turkey experiment, or in the top of the cages in the broiler breeder experiment. The first circuit (red) had peak emission in the 650-725 nm range and the second circuit (green) had peak emission between 500 to 575 nm. Before photostimulation, birds were kept under non-photostimulatory conditions i.e. 6 hr of light using both the red and the green light circuits. Hens were then photostimulated by increasing the day length to 16 hr of either the red light circuit (red group) or the green light circuit (green group). The second light circuit in each room remained at 6 hr.

In the turkey hen experiment peak egg production did not differ between the control white and red light treatment groups in weeks 5, 6, and 7 of photostimulation. Thereafter, the decline in egg production in the control birds was greater than that of the red group, and the differences between the two groups were significant in weeks 9, 14, 15, and 22 of photostimulation (P<0.05, Figs. 1 and 2). The green light group exhibited a different egg laying pattern than that of the control and red light groups following photostimulation, and their egg production remained consistently low throughout the experimental period. The overall mean egg

(Dunn and Sharp, 1999) followed by pituitary secretion of gonadotropins (Lewis et al., 2005, Lewis et al., 1998) resulting in gonad recrudescence (Sharp, 2005).

Three major sites have been shown to contain photoreceptors; the eyes (retina), the pineal gland and the deep brain tissue (ERPRs) (Foster and Soni, 1998; and Rathinam and Kuenzel, 2005). Photic cues that regulate the timing of seasonal reproductive cyclicity in birds are detected by extra-retinal photoreceptor (Kuenzel et al., 1993 and Malik et al., 2004). In contrary to the pivotal role of the pineal in mammals, enucleation and/or pinealectomy in birds caused no change in the pattern of seasonal changes of gonadal growth and secretion of luteinizing hormone (LH) (Wilson, 1991). However, covering the head so that the light cannot penetrate the skull eliminated the photoperiodic responses (Menaker et al., 1970).

Photoreceptors were suggested to be involved in the detection of daily or seasonal changes of photoperiod (Etches, 1996). All photoreceptor cells contain a large protein; an opsins, covalently bound to the aldehyde moiety of vitamin A; the chromophore (Bownds, 1967). When the chromophore absorbs a photon, the molecule isomerizes from 11-cis to all-trans configuration (Hart, 2001). This in turn leads to a conformational change in the opsin that triggers its enzymatic activity and initiates a biochemical cascade that causes a change in the rate of neurotransmitter release from the photoreceptor (Applebury and Hargrave 1986).

It has been demonstrated that domestic fowls subjected to gonad stimulating photoperiod, responded to the longer wavelengths of the spectrum (Harrison et al., 1970). The sensitivity of the bird to long wave radiation (630-780 nm) is a result of deep tissue penetration (hypothalamic ERPRs) stimulating reproductive axis (Benoit and Assenmacher, 1966 and Menaker and Underwood, 1976). In contrast, activation of retinal photoreceptors appears to cause an inhibitory effect on reproduction (Homma et al., 1972, Siopes and Wilson, 1980a,b). The response to visible radiation is probably mediated by the green-yellow bands of the light spectrum (545-575 nm), where the avian retina is in a relative peak sensitivity (Prescott and Wathes, 1999a,b).

The effects of photostimulation on hypothalamic-gonadal axis are well characterized, whereas the mechanisms that transduce relevant photic information to neuroendocrine effector neurons are not well established (Cho et al., 1998; Dawson and Goldsmith, 1997 and Peczely and Kovacs, 2000). The link between ERPRs and the reproductive axis remains an open question. Saldanha et al., (2001) reported that brain photoreceptors communicate directly with GnRH-neurons, to stimulate the reproductive axis. Additional link could be vasoactive intestinal peptide (VIP) cells, which co-localize with all opsin-expressing cells in birds (Moore et al., 1978; Opel and Proudman, 1988). The VIP within the opsins system has the potential to regulate reproductive function via synaptic interactions all along the trajectory of its axons through the lateral septum (LS) and hypothalamus.
production during the 27 weeks experimental period was significantly greater in the red light birds compared with white light control or the green light groups (Fig. 2).

Photostimulation of ERPRs (Red group) caused an elevation (P ≤ 0.05) in hypothalamic red opsin mRNA expression (Fig. 6A). Red opsin expression was also observed in the retina (Fig. 6B). On the other hand, selective photostimulation of retinal photoreceptors (Green group) caused an elevation (P ≤ 0.05) in retinal green opsin (P ≤ 0.05, Fig. 6C), whereas the expression of green opsin in the hypothalamus was very low in all groups (Fig. 6D).
effect on GnRH-I mRNA expression (Fig. 7A). A decrease ($P \leq 0.05$) in LH mRNA expression (Fig. 7C) was detected in the Green group, whereas the decrease in FSH mRNA expression was not significant (Fig. 7B).

Figure 5 - Plasma progesterone (A), testosterone (B) and estradiol (C) concentrations of Cobb broiler breeder hens of the Control group (29 lux); Red group, photostimulated with red light (29 lux) combined with non-photostimulatory green light (27.5 lux); and Green group, photostimulated with green light combined with non-photostimulatory red light. Plasma steroid concentrations were determined by enzyme-linked immunosorbent assay. Data are presented as mean ± standard error of the mean ($n = 45$). Values with different letters are significantly different ($P \leq 0.05$).

Retinal and extra-retinal photostimulation also affected the lactotrophic axis. Hypothalamic VIP mRNA expression was reduced in retinal photostimulated hens (Green group, Fig. 8A) and was positively correlated with decreased prolactin mRNA expression ($p \leq 0.05$; Fig. 8B).

These results, taken together, support previous findings that the eyes are not necessary for photic determination of reproductive stimulation. Moreover, it seems that the eyes have an inhibitory effect on reproduction, in accordance with the fact that orbital enucleation increased egg production in chickens (Harrison, 1972). In the present study, selective photostimulation with green light was accompanied with greater retinal green opsin mRNA expression. Conversely, very low hypothalamic green opsin gene expression was detected, since the ability of green light to penetrate the tissue is poor (Wan, et al., 1981). However, long wave length radiation (red band of the spectrum) has the ability to penetrate the tissue (Benoit, 1978) and therefore, we detected greater red opsin gene expression in the hypothalamus which has maximum absorbance at 630 nm (Benoit, 1978).

Figure 6 - Hypothalamic and retinal red opsin mRNA expression (A and B, respectively) and hypothalamic and retinal green opsin (C and D, respectively) of the Control group (29 lux); Red group, photostimulated with red light (29 lux) combined with non-photostimulatory green light (27.5 lux); and Green group, photostimulated with green light combined with non-photostimulatory red light. Expressions of red and green opsin were determined by real-time polymerase chain reaction. Abbreviations: A.U., arbitrary units; E-2, results×10-2. Data are presented as mean ± S.E. of the mean ($n = 5$). Values with different letters are significantly different ($P \leq 0.05$).

The role of retinal photoreceptors in reproductive activities of broiler breeder hens

The mechanism by which the eyes inhibit reproduction is unknown. The first candidate that should be investigated in relation to the inhibitory role of the eyes in reproduction is serotonin, since high levels of serotonin, which is synthesized in
by investigating the role of VIP and serotonin. Parachlorophenylalanine (PCPA) treatment, an inhibitor of serotonin (5-HT) biosynthesis, increased reproductive performances (Fig. 9) and mRNA expression of GnRH-I, LH-β and FSH-β (Fig. 10) of the Green group up to levels which did not differ from the white control group.

Serotonin may suppress the reproductive axis activity via two pathways: A. Directly through serotonin receptor type 2, 5-HT2 (Halawani and Burke, 1976) which suppresses GnRH-I synthesis (El Halawani et al., 1983) and LH secretion (Halawani and Burke, 1976). Serotonin decreases GnRH and LH release in female rats after sexual maturity (Arias et al., 1990 and Moguilovsky and Wuttke, 2001). We suggest that a similar mechanism exists also in birds, since PCPA treatment in the present study of mature broiler breeder chickens, increased GnRH-I, LH-β and FSH-β mRNA expression in agreement with the finding in female rats after sexual maturity. B. Indirectly, involving different possible modulators,
such as VIP, as prolactin-releasing hormone, (El Halawani et al., 1995) that, as mentioned before, its synthesis is controlled by serotonin (El Halawani et al., 1988; Hargis and Burke, 1984; Macnamee and Sharp, 1989). In the present study, immunization against VIP lowered prolactin mRNA expression and its plasma levels without any effect on reproductive performances. A possible explanation for this is that VIP neutralization may have decreased the direct stimulatory effect of VIP. VIP expression was demonstrated in the embryo chicken retina (Teruyama and Beck, 2001) and VIP terminals have a direct synaptic contact with GnRH cells (Van der Beek et al., 1994; Van der Beek et al., 1993 and Prada Oliveira et al., 2003). Therefore, it has been suggested that VIP may be a transducer of stimulatory photic cues to the GnRH system in birds (Saldanha et al., 1994; Saldanha et al., 2001) and that VIP neutralization should decrease reproductive axis activity. On the other hand, although PCPA treatment in the present study significantly lowered VIP mRNA expression, an elevation in reproductive performances was observed. Taken together, our study indicates that serotonin, and not VIP, is involved in the reproductive decline associated with selective retinal photostimulation.

New evidence for epigenetic manipulation in retinal opsin mRNA level

Fertilized avian eggs are usually incubated in darkness. Previous studies have shown that exposing avian eggs (e.g., broilers, White Leghorn chickens, turkeys and quails), to white or green light during incubation, increases the embryo body weight and accelerates hatching (Shultz, et al., 1962; Siegel, et al., 1969; Cooper, 1972; Walter and Voitle, 1972; Coleman and McNabb, 1975; Guatpande et al., 1995; and Shsfey and Al-Mohsen, 2002). Intermittent light regime (15 min. on and 15 min. off) enabled to study the definite effects of green light illumination on body and muscle growth in broilers and turkeys (Rozenboim et al., 2003, 2004). Combinations of light illumination in pre- and post-hatch broilers (dark vs. green light during embryonic period followed by white or green light post-hatch) revealed that the best effect on the development and growth of the chicks, is achieved when green light stimulus is provided during incubation. Taking together with recent study presented in this paper (Mobarkey, et al., 2010), that showed that in response to green light photostimulation
there was a significantly higher expression of the green opsin receptor gene, and a lower red opsin receptor gene expression in the retina, we suggested that green light, which stimulates mainly the retinal photoreceptors, apparently causes suppressed reproductive performances in adult chickens. The objective of our current study was to examine the pattern of expression of the cone photoreceptors (i.e. green and red opsins) in the retina of the developing chicken embryo during incubation. Furthermore, we tested the effect of incubation under monochromatic green or red light manipulation on the expression of the green and red opsin genes.

Findings yielded from this study, so far, show that opsin RNA transcription is first evident by Real-Time PCR technique at E14 (The 14th day of incubation). This is consistent with the findings of Bruhn and Cepko (1996) who used the in situ hybridization technique.

In Addition we found that green light during incubation suppresses the green and red opsin receptors gene expression in the last three days before hatching, while red light enhances their expression (Figures 11-12).

![Figure 11 - Green opsin mRNA gene expression in embryo retina (arbitrary units). Embryos were incubated under monochromatic light throughout the embryonic period. G.L= Green Light; R.L= Red Light; Control= dark. n=10 in each treatment group, on each sampling point (embryonic day). Results are presented as MEAN±SEM. Different letters represent a statistically significant difference (p<0.05).](image1)

![Figure 12 - Red opsin mRNA gene expression in embryo retina (arbitrary units). Embryos were incubated under monochromatic light throughout the embryonic period. G.L= Green Light; R.L= Red Light; Control= dark. n=10 in each treatment group, on each sampling point (embryonic day). Results are presented as MEAN±SEM. Different letters represent a statistically significant difference (p<0.05).](image2)

Nowadays we are working on analyzing the expression of the red and green opsins in the brains of the light-treated embryos and chicks, in order to determine the effect of light during incubation on the ERPRs as well.

Thorough and extended research is required in order to reveal the molecular mechanism by which the light affects the chicken’s genome, and the indirect way it influences its reproductive performances.
Figure 13 - Green opsin mRNA gene expression in the eye retina (arbitrary units), of embryos on days E14-E20 of incubation (represented on the graph as days -7 to -1 before hatch), and chicks from day of hatch (“0”) until 10 days of age. The embryos were incubated under Green/Red monochromatic light throughout the incubation period. Incubation in the dark was used as control. G.L= Green Light; R.L= Red Light; Control= dark. n=7 in each treatment group, on each sampling point. Results are presented as MEAN±SEM. Different letters represent a statistically significant difference (p<0.05).

Figure 14 - Red opsin mRNA gene expression in the eye retina (arbitrary units), of embryos on days E14-E20 of incubation (represented on the graph as days -7 to -1 before hatch), and chicks from day of hatch (“0”) until 10 days of age. The embryos were incubated under Green/Red monochromatic light throughout the incubation period. Incubation in the dark was used as control. G.L= Green Light; R.L= Red Light; Control= dark. n=7 in each treatment group, on each sampling point. Results are presented as MEAN±SEM. Different letters represent a statistically significant difference (p<0.05).

References


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