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Effect of Light Spectra on Growth Performance and Immune Response of Koi Carp, *Cyprinus carpio* (Linnaeus, 1758)

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Abstract

The present study was undertaken to assess the growth performance and non-specific immune response of koi carp, *Cyprinus carpio* exposed to light-emitting diodes (LEDs): blue (450 nm), green (530 nm), yellow (530 nm) and red (630 nm) under long photoperiod (16 h light) for 90 days. White fluorescent light (simulated natural photoperiod, SNP) was used as control. Growth performance of fish was higher ($p<0.05$) in blue-LED and green-LED exposed groups with significantly higher weight gain percentage, FER and SGR and lower FCR. The concentrations of stress indicators: (cortisol and glucose) were significantly ($p<0.05$) higher in yellow-LED, red-LED and for control fish group. Total protein, globulin protein, lysozyme activity and respiratory burst activity was significantly higher ($p<0.05$) in blue-LED and green-LED exposed fish groups whereas, yellow-LED and red-LED exposed fish recorded decline in this parameters after 60 days. The present study recommends that blue and green lights are suitable for better growth and improving the non-specific immune system of koi carp.

Keywords: light spectra, light emitting diodes, growth, immune response, koi carp

Introduction

Light is the most important factor that controls circadian rhythms and many of the physiological and behavioral changes in fishes (Pierce et al., 2008). This effect of light varies based on the light-quality (spectra or wave length), quantity (intensity), periodicity (photo period), water absorbance properties as well as the specific light sensitivities of the species being reared. Entire life cycle of fish, starting from embryo development (Downing & Litvak, 2001) to sexual maturation (Migaud et al., 2010) is profoundly affected by light.

Among these characteristics, periodicity is the most studied factor and very few studies have been conducted on the effect of wavelength of light on physiological performance of fishes. Fishes are capable of detecting differences in the intensity and spectra of light by retinal and extra-retinal photoreceptors (Bayarri et al., 2002; Vera et al., 2010). Sensitivity to these differences varieys among species; and affect behaviour, somatic growth, reproductive activity and the survival of juveniles and larvae (Ruchin, 2004; Bapary et al., 2011)

Light emitting diodes (LEDs) produce light of specific wavelengths (Migaud et al., 2007). The LED light has lower power requirements, electrical running costs, and a longer life span than the standard metal halide bulbs (Migaud et al., 2007), so LED lights could provide much more efficient lighting systems than those currently used in the fish farming industry because they can be tuned to a species specific environmental sensitivity by emitting narrow bandwidths (Villamizar et al., 2009).

It was recently reported by Shin et al., 2011 that green and blue LEDs with short wavelengths, increased antioxidant activity in yellowtail clownfish, *Amphiprion clarkia*. The blue wavelength has also been shown to play a role in protection against stress in the Nile tilapia, *Oreochromis niloticus* (Volpat & Barreto, 2001). The red LED wavelength affects physiological function and induces oxidative stress in yellowtail clownfish (Shin et al., 2011).

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Above studies indicate that LEDs could be applied to induce a photo environment among various inhabitation environment factors. Very few studies have been conducted on stress and immune response of fish to light wavelengths (Head & Malison, 2000; Van der Salm et al., 2004; Shin et al., 2011; Choi et al., 2012).

The present study was undertaken to analyse the effect of different LED light spectra under long photoperiod (16 L: 8 D) on the growth, stress level and immunological response of Koi Carp (*Cyprinus carpio*)

Materials and Methods

Koi Carp, *Cyprinus carpio* (n=300, average weight 16.24 ± 0.243 g and aged 3 months) were procured from the local market (Mumbai, Maharastra, India). These fishes were stocked in fifteen rectangular shaped, white colour (inner side) tanks (100 L capacity) fitted with proper aeration and circulation of water through separate inlet-outlet pipes (five treatments with 3 replicates) by following randomized selection design (n=20 fishes/tank). Fishes were allowed for 2 weeks acclimatization, during which the fish were exposed to light from a white fluorescent bulb (27 W). The control group was also exposed to white light (Simulated Natural Photoperiod, SNP Group). The light intensity near the water surface of the tank was 0.96 W/m^2. During the experiment water temperature was 25±1^{o}C (monitored weekly twice) and a long photoperiod (16 h Light: 8 h Dark period) was provided through LED bulbs. Electronic timers were used to control lighting system automatically. The fishes were exposed to Blue-LED (peak at 450 nm), Green-LED (peak at 530 nm), Yellow-LED (peak at 580 nm) and Red-LED (peak at 630 nm) procured from Dasin LED Co. kyunggi, Korea. The LEDs were set 30 cm above the surface of water and irradiance at the surface of water was 0.9 W/m^2.

Fishes were reared for 90 days and sampling was done monthly on 30th day, 60th day and 90th day. Commercial feed containing 34.5% protein was given throughout the experiment. The fishes were fed with commercial feed twice daily (@ 3.5% of body weight in first 60 days and 3.0% of the body weight during remaining period. The water quality parameters such as dissolved oxygen (5.5 ± 0.01 mg L^{-1}), ammonia (0.12± 0.01 mg L^{-1}), nitrites (0.05 ± 0.004 mg L^{-1}), hardness (220.6 ± 15.10 mg L^{-1}) and pH (7.6 ± 0.3) were in optimum range (monitored weekly) during the experiment period.

To estimate the fish growth, fish weight was recorded at the startof the experiment and after that, every 30 days interval, till the end of the trial. The fishes were starved for 24 h before handling and anaesthetized using 50 μL^{-1} of clove oil (Sudagar et al., 2009) for sample collection and biometric procedures. Daily feed intake and growth parameters such as percentage weight gain (WG%), specific growth rate (SGR), feed conversion ratio (FCR), Feed efficiency ratio (FER) were also calculated as follows (Wangmi et al., 2009)

$$\text{Percentage weight gain (WG \%)} = \frac{[(\text{Final Weight}) - (\text{Initial Weight}] \times 100}{\text{Initial Weight}}$$

$$\text{Specific growth rate} = \frac{[\ln(\text{Final Weight}) - \ln(\text{Initial Weight}] \times 100}{\text{Experimantal periods in days}}$$

$$\text{Food conversion ratio} = \frac{\text{Feed Given (g)}}{\text{Weight Gain (g)}}$$

$$\text{Feed efficiency ratio} = \frac{\text{Weight Gain (g)}}{\text{Feed Given (g)}} \times 100$$

The blood samples were collected every month to assess nitrobluetetrazolium (NBT) activity. Two fish from each replicate tank (6 fishes from each treatment) were gently caught and anaesthetized (with clove oil, 50 μL^{-1}). Blood samples (0.5-0.8 mL) were collected by caudal vein puncture using 2 mL heparinized syringes and immediately transferred to test tubes containing EDTA powder (as an anticoagulant) and shaken gently to prevent hemolysis of blood.

For serum collection two fishes from each replicate tank (6 fishes from each treatment) were anaesthetized and blood was collected without anticoagulant and allowed to clot for 2 h, followed by the collection of straw coloured serum with micropipette and stored at -20^{o}C until use. The serum was used for the estimation of serum protein, albumin, glucose level and cortisol level. The serum samples were stored at -70°C until analysis of immunological and stress indicators.

The estimation of total protein and albumin concentration were carriedout using total protein kit, (Biuret method) and albumin kit, (BCG dye binding method) of Merck, Germany respectively. Globulin content was calculated by subtracting albumin values from total serum protein (Kumar et al., 2005).

The lysozyme activity was measured using the turbidity assay. Chicken egg lysozyme (Sigma) was used as standard and 0.2 mg mL^{-1} lyophilized *Micrococcus lysodeikticus* (Bangalore Genei, India) in 0.04 M sodium phosphate buffer (pH 5.75) was used as substrate. Lysozyme activity was expressed as U/min. Nitrobluetetrazolium (NBT) assay was carried at by modified Stasiack and Bauman method (Stasiack & Bauman, 2006).

The OD was read in micro plate reader (BioTek Power Wave 340, India) at 620 nm. Cortisol content in serum was assayed with Cayman's Cortisol EIA Kit (Cayman's Chemical, USA). The detection limit for this EIA kit was 35 pg mL^{-1}. Glucose level in serum samples were measured using the Cayman's Cortisol EIA Kit (Cayman's Chemical, USA).

One-way analysis of variance (ANOVA) was carried out using SPSS (Version 16.0, Chicago, IL, USA), Duncan's multiple range test (Duncan, 1955) was used for post-hocanalysis. All experimental data were expressed as mean±SE.

Results and Discussion

No fish mortality was observed during the experiment. There was no significant difference ($p>0.05$) in the mean weight of fish among the treatments on 30th day of sampling (Fig. 1). After that, growth performance of koi carp was significantly influenced by LED light spectrum. Fishes from blue-LED and green-LED groups recorded higher mean weight and differed significantly from theother groups. Growth performance related data at the end of experiment (90th day) is shown in Table 1. FBW, WG (%) and SGR significantly increased in fishes reared under green LED spectrum on 90th day. Fishes from green-LED group showed the highest mean weight during the experiment whereas fishes from red-LED and SNP groups showed the lowest mean weight. FCR and FER were significantly better ($p<0.05$) in green-LED fish group.

The response of fish to the light colour (wavelength) is species specific (Ruchin et al., 2002) and koi carp also showed better growth like silver carp, common carp and rainbow trout (Radenko & Alimov, 1991; Ruchin et al., 2002; Luchari & Pirhonen, 2008). It was reported that green and blue light enhance GH (growth hormone) levels and melaton, which plays a role in modulating growth of the yellowtail clownfish, *Amphiprion clarkia* (Shin et al., 2012).

Table 1. Growth performance of Koi carp, *Cyprinus carpio*, reared under different LEDs light spectra for 90 days (mean±SE)[1].

Treatment	WG (%)	FCR	SGR
SNP	$122.460^{a}\pm9.246$	$3.333^{b}\pm0.235$	$0.884^{a}\pm0.045$
Blue-LED	$176.431^{bc}\pm5.025$	$2.513^{a}\pm0.090$	$1.129^{bc}\pm0.021$
Green-LED	$200.137^{c}\pm14.134$	$2.323_{a}\pm0.106$	$1.215^{c}\pm0.052$
Yellow-LED	$147.392^{ab}\pm19.203$	$2.881^{ab}\pm0.279$	$0.989^{ab}\pm0.090$
Red-LED	$120.106^{a}\pm7.697$	$3.197^{b}\pm0.149$	$0.873^{a}\pm0.038$

[1]WG (%), Weight gain percentage; FCR, feed conversion ratio and SGR, specific growth rate. Different superscripts in a column indicate significant differences ($p< 0.05$) between the treatments. SNP -Simulated natural photoperiod, LEDs.

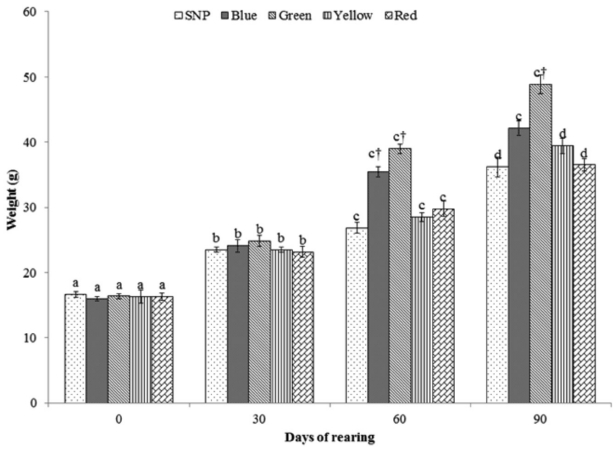

Fig. 1. The growth performance of Koi carp, *Cyprinus carpio*, reared under blue, green, yellow, red LEDs and simulated natural photo period for 90days. The fishes were reared under long photoperiod (16L: 8D). Letters indicate significant differences among sampling days within the same light spectra ($p<0.05$). The crosses (†) indicate significant differences among light spectra on the same sampling day ($p<0.05$). Data are presented as mean± SE (n= 30).

LED light spectrum seemed to influence serum cortisol levels. SNP, yellow-LED and red-LED fish groups (exposed) had significantly higher cortisol level ($p<0.05$) compared to other groups on 30th day (Fig. 2b). On 60th day cortisol level decreased significantly in fishes from SNP, yellow-LED and red-LED groups compared to 30th day from the same spectrum fish group, but there after there was no significant decrease ($p>0.05$) in cortisol level on 90th day. Here blue-LED and green-LED fish groups reported low level of cortisol during the experiment. Glucose level in SNP and red-LED fish groups was significantly higher ($p<0.05$) than other treatments on 30th day and there was continuous decrease in glucose level upto the end of experiment. Plasma glucose level varied from 166.68 ± 2.981 to 41.369 ± 2.337 mgdL^{-1}and fishes from blue-LED and green-LED groups showed significant low glucose level during the experiment (Fig. 2a).

High stress level in red-LED group fishes similar to that reported by Shin et al. (2011) who found red light as oxidative stress inducer in yellowtail clownfish (*Amphiprion clakii*). In previous studies it was reported that green and blue lights inhibit oxidative stress in Nile tilapia, *Oreochromis niloticus* (Volpato & Barreto, 2001) and Cinnamon clownfish, *(Amphiprion menanopus*) (Choi et al., 2012). This variation in stress indicators response could be due to the response of the pineal gland to different wavelengths. The pineal gland and eyes of fish could modulate the secretion of melatonin hormone, α-melanocyte-stimulating hormone (αMSH) and melanin-concentrating hormone (MCH) which in turn stimulate cortisol release and stress response from hypothalamus–pituitary–interrenal (HPI) axis (Lamers et al., 1992; Rotllant et al., 2003). Fishes exposed to yellow-LED and red-LED along with SNP reported decrease in cortisol and glucose level after 60th day which could be due to the preventive role of increased melatonin in these groups that reduce oxidative stress.

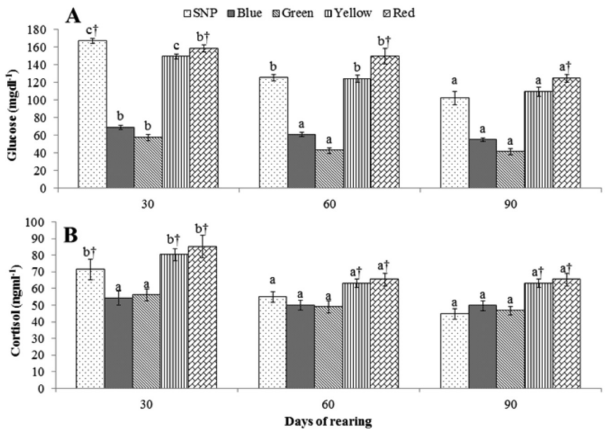

Fig. 2. Serum Glucose (A) and Serum cortisol (B) levels of Koi carp, *Cyprinus carpio* reared under blue, green, yellow, red LEDs and simulated natural photoperiod for 90 days.

NBT level did not vary significantly ($p>0.05$) during this trial except thered-LED fish exposed groups. There was continuous decrease in NBT level in red-LED groups during this study. In blue-LED and green-LED fish groups NBT levels were significantly ($p<0.05$) higher as compared to other spectrum groups. NBT levels were highest in green-LED fish groups (0.5382 ± 0.047) and lowest in red-LED fish groups (0.264 ± 0.025) on 60th day of the experiment (Fig. 3b).

Total protein, albumin and globulin were not significantly different among the different spectrum groups on 30th day but, after that these proteins presented different trends (Fig. 4). Total protein and globulin increased continuously but albumin increased upto 60th day and after that it decreased in blue-LED and green-LED fish groups. Immune

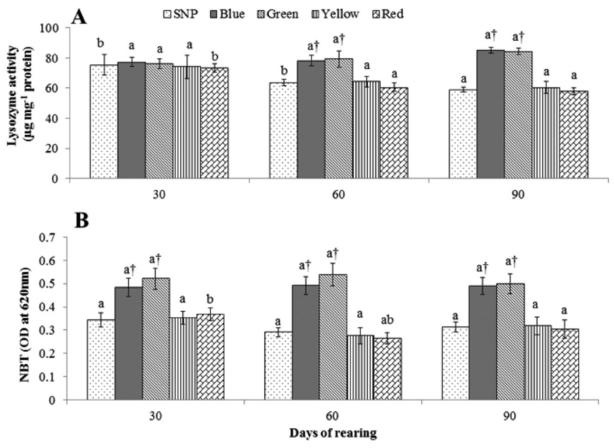

Fig. 3. Serum lysozyme activity (A) and serum nitrobluetetrazolium activity (NBT) levels of Koi carp, *Cyprinus carpio* reared under blue, green, yellow, red LEDs and simulated natural photoperiod for 90 days. The fish were reared under long photoperiod (16L: 8D). Letters indicate significant differences among sampling days within the same light spectra ($p<0.05$). The crosses (†) indicate significant differences among light spectra on the same sampling day ($p<0.05$). Data are presented as mean ± SE.

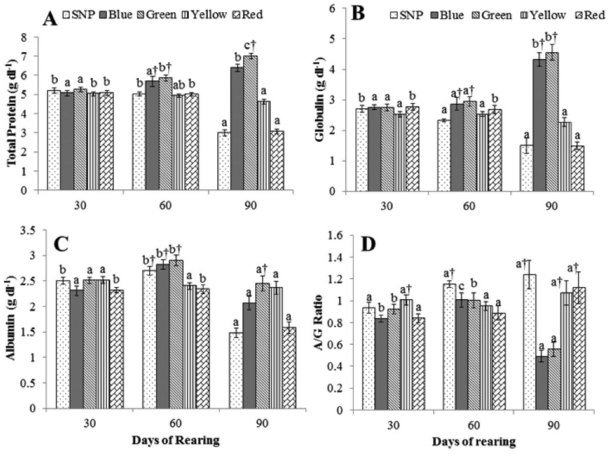

Fig. 4. Immune proteins of Koi carp, *Cyprinus carpio* reared under blue, green, yellow, red LEDs and simulated natural photoperiod for 90 days. (A) : Total Proteins, (B) : Globulins (C) Albumin (D): Albumin: Globulin ratio. Letters indicate significant differences among sampling days within the same light spectra ($p<0.05$). The crosses (†) indicate significant differences among light spectra on the same sampling day ($p<0.05$). Data are presented as mean ± SE

proteins decreased significantly ($p>0.05$) after 30th day in fish from red-LED group compared to other spectrum groups. A: G ratio varied among the spectrum groups and lowest A: G ratio was found in blue-LED and green-LED fish groups at the end of experiment.

Results showed that there was significant decline in lysozyme activity, NBT levels and immune proteins in SNP, yellow-LED and red-LED fish groups which may be due to chronic stress in these spectrum groups. In previous studies, prolonged secretion of cortisol in fish subjected to chronic stress was known to be a strong immunosuppressive agent (Tort, 2011). Cortisol and immune indicators change concurrently after repeated sampling stress in trout causing a reduction of immune competence (Sunyer & Tort, 1995). Severe immune suppression in cold-shock affected sea bream is correlated with high levels of cortisol (Tort et al., 1998). Higher level of stress results in reduction in levels of complement together with high cortisol in sea bream (Montero et al., 1999; MacKenzie et al., 2006) and red porgy (Rotllant & Tort, 1997).

The improvement in total proteins and globulins in green-LED group could be due to enhanced immunity of koi carp indicated by enhanced lysozyme activity and lower cortisol levels (less stress) in the same group. The increse in lysozyme activity in green-LED group supported by the study of Choi et al., 2012 who reported significant increment of lysozyme activity in green-LED group Cinnamon clownfish, *Amphirpion melanopus*.

Based on above results, it can be concluded that blue and green LED lights can be used for better growth of koi carp. This study also found the negative effect of long wavelength lights on immune proteins and lysozyme activity. However, it was also observed that blue and green colour light not only help to maintain lower stress levels but also enhance the immunity of koi carp. Additional studies are needed to identify the mechanism of protective capacity using photoreceptors and various stress factors in fish.

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