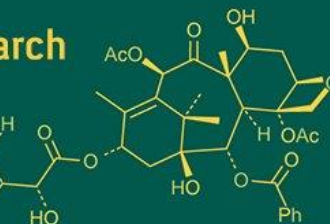
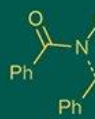


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Effect of different colors (Red, Blue, Green and White) of artificial light on pigmentation, growth performance, feed utilization and immune response of tilapia (*Oreochromis niloticus*)

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Abstract

The present study investigated the effects of different artificial light colors on growth, pigmentation, feed utilization, survival, and immune response of Nile tilapia (*Oreochromis niloticus*) over a 60-day period under controlled laboratory conditions. The experiment was conducted at the Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval, using a Completely Randomized Design (CRD) comprising five treatments and four replicates. Fish were exposed to natural light (control) and red, blue, green, or white LED light for 12 hours during the night. Significant differences were observed among treatments. Fish reared under green light exhibited the highest final weight (25.22 ± 0.138 g), weight gain (25.72 ± 0.138 g), specific growth rate ($6.53 \pm 0.030\%$), feed efficiency ratio (0.563 ± 0.003), protein efficiency ratio (1.88 ± 0.011), and survival rate (100%). Blue light resulted in the highest carotenoid content ($5.62 \pm 0.035 \mu\text{g g}^{-1}$) and enhanced immune status, as reflected by elevated RBC and WBC counts. The findings indicate that green light promotes growth and feed utilization, whereas blue light improves pigmentation and immunity, making both suitable for enhancing tilapia production and health.

Keywords: *Oreochromis niloticus*, artificial light color, growth performance, pigmentation, immune response

1. Introduction

Aquaculture is one of the fastest-growing food production sectors globally and plays a vital role in ensuring food security, nutritional sustainability, and economic development. Among cultured fish species, Nile tilapia (*Oreochromis niloticus*) is widely recognized for its rapid growth, high tolerance to diverse environmental conditions, disease resistance, and excellent market demand (El-Sayed, 2019) [4]. Consequently, optimizing culture conditions to enhance growth performance, feed utilization, health status, and product quality has become an important focus of aquaculture research.

Light is one of the most influential environmental factors affecting fish physiology and behavior. In addition to regulating vision, light influences feeding activity, growth, reproduction, stress response, pigmentation, and immune function through endocrine and metabolic pathways (Boeuf & Le Bail, 1999) [3]. Fish perceive different wavelengths of light through specialized photoreceptors, and variations in light spectrum can induce distinct physiological and behavioral responses. Recent advances in light-emitting diode (LED) technology have enabled precise manipulation of light quality in aquaculture systems, providing opportunities to improve production efficiency through environmental control. Several studies have demonstrated that light color significantly affects growth performance and feed utilization in fish. Green and blue wavelengths have frequently been associated with enhanced growth, improved feed conversion efficiency, and reduced stress in cultured species (Ruchin, 2004; Luchiari & Freire, 2009) [10, 7]. Similarly, the use of specific light spectra has been reported to influence survival and physiological performance by modulating metabolic activity and endocrine regulation. However, the response to different light colors varies among species and developmental stages, necessitating species-specific investigations.

Pigmentation is another important quality attribute in aquaculture, particularly because coloration influences consumer preference and market value. Carotenoids are responsible for coloration in fish and play essential roles as antioxidants and immune enhancers. Environmental factors, including light spectrum, can affect carotenoid deposition and pigmentation intensity in fish tissues (Noureldin *et al.*, 2021)^[8]. Furthermore, recent studies suggest that light color may influence hematological parameters and immune responses by affecting stress levels and physiological homeostasis.

Despite increasing interest in the application of colored artificial lighting in aquaculture, information regarding its effects on growth performance, feed utilization, pigmentation, and immune response in Nile tilapia remains limited. Therefore, the present study was undertaken to evaluate the effects of red, blue, green, and white artificial light on growth, survival, feed utilization efficiency, pigmentation, and hematological parameters of *Oreochromis niloticus* under controlled culture conditions. The findings of this study may contribute to the development of sustainable and cost-effective lighting strategies for improving tilapia production and overall aquaculture productivity.

2. Materials and Methods

2.1 Experimental Site and Duration

The experiment was conducted for 60 days from 1 October to 29 November 2025 at the Experimental Hands-on Training Centre, Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India. The study was designed to evaluate the effects of different colors of artificial light on growth performance, feed utilization, pigmentation, survival, and immune response of Nile tilapia (*Oreochromis niloticus*).

2.2 Experimental Fish and Rearing Conditions

Healthy Nile tilapia fingerlings were procured from a local hatchery and acclimatized to laboratory conditions for 2-3 days before the commencement of the experiment. A total of 240 fish with an average initial body weight of 0.51 ± 0.01 g were randomly distributed into twenty plastic tanks ($2 \times 1 \times 1$ ft; 50-L capacity). Each tank was stocked with 12 fish and maintained with 30-35 L of dechlorinated freshwater.

Continuous aeration was provided using air stones connected to a centralized aeration system. Approximately 10% of the water volume was exchanged daily to maintain water quality. Water quality parameters were monitored regularly and maintained within the optimum range for tilapia culture.

2.3 Experimental Design and Light Treatments

The experiment was conducted using a Completely Randomized Design (CRD) comprising five treatments with four replicates each. The treatments included:

- **T₀:** Control (natural light)
- **T₁:** Provide red light for 12 hour during night time
- **T₂:** Provide blue light for 12 hour during night time
- **T₃:** Provide green light for 12 hour during night time
- **T₄:** Provide white light for 12 hour during night time

Colored LED lights were installed above the respective tanks and operated for 12 h during the night throughout the experimental period. Light intensity was maintained

uniformly across all treatments to eliminate intensity-related effects.

2.4 Diet Preparation and Feeding

A formulated diet containing 30% crude protein was prepared using fish meal, groundnut oil cake, wheat flour, tapioca powder, fish oil, sunflower oil, and vitamin-mineral premix (AOAC, 2019). The ingredients were finely ground, thoroughly mixed, pelletized, and dried before use.

Fish were fed twice daily at 09:00 and 18:00 h. The feeding rate was maintained at approximately 2-3% of body weight, and feed quantity was adjusted at 15-day intervals based on growth measurements (Proximate composition of feed ingredients and Composition of experimental diet given in table 1).

2.5 Growth Performance Assessment

Fish were sampled every 15 days to evaluate growth performance. Body weight was recorded using a digital balance, and growth indices were calculated using standard equations:

$$\text{Weight Gain (WG)} = \text{Final Weight} - \text{Initial Weight}$$

$$\text{Percentage Weight Gain (\%)} = \frac{(\text{Final Weight} - \text{Initial Weight})}{\text{Initial Weight}} \times 100$$

$$\text{Specific growth rate (\%)} = \frac{\ln [(\text{Final body weight})] - \ln [(\text{Initial body weight})]}{\text{Experimental days}} \times 100$$

Where,

W₁ = initial weight

W₂ = ultimate weight

T = experiment duration

$$\text{Survival Rate (\%)} = \frac{(\text{Number of Fish Survived})}{(\text{Number of Fish Stocked})} \times 100$$

2.6 Feed Utilization Parameters

Feed utilization efficiency was assessed at the end of the experiment through the following indices:

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed Intake (g)}}{\text{Weight Gain (g)}}$$

$$\text{Feed Efficiency Ratio (FER)} = \frac{\text{Weight Gain (g)}}{\text{Feed Intake (g)}}$$

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Weight Gain (g)}}{\text{Protein Intake (g)}}$$

2.7 Pigmentation Analysis

The total carotenoid content was determined following the method described by Olson *et al.* (1979)^[9], which is commonly used to estimate carotenoid levels in different tissues. After the feeding trial, carotenoid concentrations were analyzed in the skin, muscle, and whole body of the fish.

For the analysis, 1 g of Tilapia muscle and skin tissue was collected and placed in a 10 ml screw-cap glass vial, where it was gently homogenized using a glass rod. To facilitate extraction, 2.5 g of anhydrous sodium sulfate was added, followed by the addition of 5 ml chloroform. The vial was then sealed and kept at 0°C overnight for proper extraction of pigments.

The carotenoid content was quantified based on the wavelength of maximum absorption. For optical density

measurement, 0.3 ml of the chloroform extract was diluted to 3 ml with absolute ethanol, and absorbance was recorded at wavelengths of 500 nm. Readings were taken after a clear 1-2 cm chloroform layer formed above the residue, and values were compared with a similarly prepared blank. The final carotenoid concentration was calculated using the highest absorbance value obtained.

Total Carotenoid concentration (μg)

$$= \frac{\text{Absorbance at maximum wavelength} \times 10}{0.25 \times \text{sample weight in grams}}$$

Where,

10 = Dilution factor

0.25 = Extinction coefficient

2.8 Immune Response Assessment

At the end of the experimental period, blood samples were collected from randomly selected fish from each treatment. Blood was drawn from the caudal vein using sterile syringes and analyzed for hematological parameters. Red blood cell (RBC) and white blood cell (WBC) counts were determined using standard hematological methods to evaluate immune status.

The red blood cell (RBC) count was determined following the method described by Lal (2010)^[6]. Blood samples were diluted at a ratio of 1:200 using RBC diluting fluid (Hayem's fluid). Fresh blood was drawn into an RBC pipette up to the 0.5 mark and then diluted with Hayem's fluid up to the 101 mark. The contents were mixed thoroughly within the pipette to ensure uniform distribution of cells.

A small amount of the diluted sample was then loaded into a haemocytometer, and the cells were counted under a high-power microscope. Counting was carried out in five small squares of the central grid four at the corners and one in the center.

The total number of RBCs was calculated using the following formula:

$$\text{No. of RBC/mm}^3 = \frac{(\text{Number of cells counted} \times \text{dilution (1:200)})}{\text{Area counted (5 squares)} \times \text{depth (0.1)}}$$

The white blood cell (WBC) count was estimated according to the method of Shah and Altindag (2004). Blood samples were diluted in a 1:20 ratio using Turk's fluid. The diluted blood was drawn into a WBC pipette, mixed properly, and then introduced into a haemocytometer for counting.

Cell counting was performed under high magnification, focusing on the four large corner squares of the counting chamber.

The total number of WBCs was calculated using the formula:

$$\text{No. of WBC/mm}^3 = \frac{(\text{Number of cells counted} \times \text{dilution (1:20)})}{\text{Area counted (4 squares)} \times \text{depth (0.1)}}$$

2.9 Analysis of Water Quality Parameters

Throughout the experimental period, key water quality parameters such as temperature, pH, and dissolved oxygen were monitored on a weekly basis. Temperature was measured using a thermometer, while pH was recorded with a pH meter. Dissolved oxygen were analyzed using titration

methods. Dissolved oxygen (DO), which reflects the amount of free oxygen available in water, was estimated using the Winkler method. This method involves the use of reagents such as manganous sulphate (Winkler A), a mixture of potassium iodide and sodium hydroxide (Winkler B), concentrated sulfuric acid, starch indicator, and 0.025 N sodium thiosulfate solution (APHA, 2017).

2.10 Statistical Analysis

All experimental data were expressed as mean $\pm$ standard error (SE). Statistical analysis was performed using one-way analysis of variance (ANOVA) under a Completely Randomized Design. Differences among treatment means were considered significant at $P < 0.05$.

3. Results

The growth performance of *Oreochromis niloticus* was significantly affected by different colors of artificial light. At the end of the 60-day experimental period, fish exposed to green light (T_3) attained the highest final weight (25.22 ± 0.138 g), followed by blue light (T_2) (22.25 ± 0.049 g). Fish maintained under natural light (T_0), red light (T_1), and white light (T_4) recorded significantly lower final mean weights.

Similarly, weight gain was highest in T_3 (25.72 ± 0.138 g), followed by T_2 (22.75 ± 0.049 g) as shown in table 2 and graphical representation shown in figure 1. Percentage weight gain ranged from $3835.44 \pm 38.63\%$ to $4946.35 \pm 90.99\%$, with the maximum value observed in T_3 and the minimum in T_4 .

Specific Growth Rate (SGR) also differed significantly among treatments. Fish reared under green light recorded the highest SGR ($6.53 \pm 0.030\%$), followed by blue light ($6.31 \pm 0.029\%$), whereas lower values were observed in T_0 , T_1 and T_4 (Table 3).

Significant differences in survival were observed among treatments. Green light treatment (T_3) achieved 100% survival throughout the experimental period, followed by blue light treatment (T_2) with $97.92 \pm 2.08\%$ survival. The lowest survival rate was recorded under red light (T_1) ($72.92 \pm 3.99\%$), while T_0 and T_4 showed survival rates of $79.17 \pm 2.41\%$ (Table 3).

Feed utilization parameters were significantly influenced by light color. The lowest Feed Conversion Ratio (FCR) was recorded in T_3 (1.78 ± 0.0105), followed by T_2 (1.80 ± 0.0013). Higher FCR values were observed in T_0 (1.82 ± 0.0088), T_1 (1.81 ± 0.0054), and T_4 (1.82 ± 0.0090) (Table 3).

The highest Feed Efficiency Ratio (FER) was recorded in T_3 (0.563 ± 0.00334), while T_0 exhibited the lowest value (0.549 ± 0.00266). Protein Efficiency Ratio (PER) ranged from 1.83 ± 0.0089 to 1.88 ± 0.0111 , with the highest value observed in T_3 (Table 3).

Carotenoid content increased progressively throughout the experimental period in all light-treated groups. At the end of 60 days, fish exposed to blue light (T_2) exhibited the highest carotenoid concentration ($5.62 \pm 0.035 \mu\text{g g}^{-1}$), followed by green light (T_3) ($5.43 \pm 0.019 \mu\text{g g}^{-1}$) and white light (T_4) ($5.04 \pm 0.023 \mu\text{g g}^{-1}$) (visual result shown in figure 2). The lowest carotenoid concentrations were observed in the control (T_0) and red light (T_1) treatments as shown in table 4 and graphical representation shown in figure 1.

Artificial light color significantly affected hematological parameters. Fish reared under blue light exhibited the highest red blood cell (RBC) count ($1.65 \pm 0.005 \times 10^6$

mm⁻³) and white blood cell (WBC) count ($13.20 \pm 0.061 \times 10^3$ mm⁻³), followed by green light treatment. Lower values were observed in fish maintained under red light and natural light conditions (Table 5).

Throughout the 60-day experimental period, the physico-chemical parameters of water remained within suitable ranges for the culture of *Oreochromis niloticus*. Water temperature varied from 25.25 to 28.50°C, while dissolved oxygen concentrations ranged between 4.50 and 5.00 ppm. The pH values remained stable between 7.20 and 8.00, indicating a slightly alkaline environment favorable for tilapia growth. Total hardness ranged from 180 to 240 ppm. No significant differences ($p > 0.05$) were observed among treatments for any water quality parameter, indicating that all experimental tanks maintained uniform environmental conditions throughout the study. Therefore, the differences observed in growth performance, feed utilization, pigmentation, survival, and immune response could be attributed primarily to the effects of artificial light color rather than variations in water quality.

4. Discussion

Light is an important environmental factor that influences fish physiology, behavior, metabolism, and growth. In the present study, significant improvements in growth performance were observed under green and blue light conditions. Fish exposed to green light exhibited superior final weight, weight gain, percentage weight gain, and specific growth rate compared with all other treatments. The improved growth under green light may be attributed to enhanced feeding activity, better visual perception of feed, and reduced stress levels. Green wavelengths penetrate water efficiently and provide a favorable environment for feeding and growth. Similar findings were reported by Luchiaro *et al.* (2008) [7], who observed enhanced growth performance under green light conditions. Ruchin (2004) [10] also reported superior growth under green and blue wavelengths compared with red light.

The higher survival observed under green and blue light treatments suggests that these wavelengths provide a more suitable culture environment for tilapia. Light color influences stress-related endocrine responses and behavioral

patterns. Reduced stress may contribute to improved health status and survival. Similar observations have been reported in several fish species where green and blue light promoted better welfare and survival than red light.

Feed utilization efficiency was significantly enhanced under green light, as evidenced by lower FCR and higher FER and PER values. Improved feed conversion indicates more efficient utilization of nutrients for growth. Enhanced feeding behavior and metabolic efficiency under green light may have contributed to these results. Elsbaay (2013) [5] reported improved feed utilization in Nile tilapia cultured under selected LED light wavelengths, supporting the findings of the present study.

Pigmentation was significantly influenced by light spectrum. Blue light produced the highest carotenoid content, indicating enhanced pigment deposition and retention. Light wavelength affects chromatophore activity and carotenoid metabolism, thereby influencing fish coloration. Improved pigmentation under blue light has also been reported by Noureldin *et al.* (2021) [8]. Since body coloration is an important quality attribute affecting consumer preference and market value, the use of blue light may provide additional economic benefits in tilapia culture. Hematological parameters demonstrated that blue light enhanced immune response. Fish exposed to blue light recorded the highest RBC and WBC counts, suggesting improved physiological condition and immune competence. Elevated RBC levels indicate improved oxygen transport capacity, while increased WBC counts reflect enhanced disease resistance. Blue light has been reported to influence neuroendocrine pathways associated with stress reduction and immune regulation, which may explain the improved hematological profile observed in the present study.

Overall, the results indicate that artificial light color can be effectively manipulated to improve tilapia performance. Green light was most effective in promoting growth, feed utilization, and survival, whereas blue light enhanced pigmentation and immune response. These findings demonstrate the potential application of colored LED lighting as a sustainable management strategy for improving productivity and fish quality in aquaculture systems.

Table 1: Proximate composition of feed ingredients (%) and Composition of experimental diet

Ingredients	Crude protein (%)	Crude-fat (%)	Ash (%)	Moisture (%)
Fish meal	53.57	4.80	14.90	7.83
GNOC	34.67	4.61	8.36	7.39
Wheat flour	10.50	1.10	5.28	7.67
Tapioca flour	2.00	0.54	0.18	11.29

Ingredients (%)	Diets (30% Protein)				
	T ₀ Control	T ₁	T ₂	T ₃	T ₄
Sterilized Fish meal	38.00	38.00	38.00	38.00	38.00
GNOC	28.00	28.00	28.00	28.00	28.00
Wheat flour	17.00	17.00	17.00	17.00	17.00
Tapioca powder	11.00	11.00	11.00	11.00	11.00
Fish oil	2.00	2.00	2.00	2.00	2.00
Sun flower oil	2.00	2.00	2.00	2.00	2.00
Vitamin and minerals premix	2.00	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00	100.00

Table 2: Weight gain (g) of Tilapia cultured with different colors (red, blue, green and white) of artificial light (Mean $\pm$ SE)

Treatment	Days				
	0 Days	15 Days	30 Days	45 Days	60 Days
T ₀	0.51 $\pm$ 0.008a	3.91 $\pm$ 0.026c	8.36 $\pm$ 0.046c	13.76 $\pm$ 0.042c	20.44 $\pm$ 0.115c
T ₁	0.51 $\pm$ 0.005a	3.92 $\pm$ 0.029c	8.32 $\pm$ 0.130c	13.43 $\pm$ 0.154d	20.50 $\pm$ 0.102c
T ₂	0.52 $\pm$ 0.009a	4.32 $\pm$ 0.039b	9.56 $\pm$ 0.050b	16.76 $\pm$ 0.092b	22.75 $\pm$ 0.049b
T ₃	0.51 $\pm$ 0.007a	5.06 $\pm$ 0.083a	10.22 $\pm$ 0.087a	18.72 $\pm$ 0.065a	25.72 $\pm$ 0.138a
T ₄	0.52 $\pm$ 0.005a	4.08 $\pm$ 0.084c	8.46 $\pm$ 0.052c	13.87 $\pm$ 0.067c	20.56 $\pm$ 0.075c

Table 3: Growth parameters analysis of tilapia cultured with different colors (red, blue, green and white) of artificial light (Mean $\pm$ SE)

Parameters	Control (T ₀)	T ₁	T ₂	T ₃	T ₄
SGR	6.16 $\pm$ 0.023c	6.16 $\pm$ 0.022c	6.31 $\pm$ 0.029b	6.53 $\pm$ 0.030a	6.12 $\pm$ 0.016c
Survival Rate	79.17 $\pm$ 2.41b	72.92 $\pm$ 3.99b	97.92 $\pm$ 2.08a	100 $\pm$ 0.00a	79.17 $\pm$ 2.41b
FCR	1.82 $\pm$ 0.0088b	1.81 $\pm$ 0.0054b	1.80 $\pm$ 0.0013b	1.78 $\pm$ 0.0105a	1.82 $\pm$ 0.0090b
FER	0.549 $\pm$ 0.003b	0.554 $\pm$ 0.002b	0.554 $\pm$ 0.0004b	0.563 $\pm$ 0.003a	0.551 $\pm$ 0.003b

Table 4: Pigmentation content of Tilapia cultured with different colors (red, blue, green and white) of artificial light (Mean $\pm$ SE)

Treatment	Days				
	0 Days	15 Days	30 Days	45 Days	60 Days
T ₀	3.45 $\pm$ 0.017ab	3.33 $\pm$ 0.019d	3.22 $\pm$ 0.035d	2.97 $\pm$ 0.081c	2.83 $\pm$ 0.068d
T ₁	3.43 $\pm$ 0.014b	3.33 $\pm$ 0.019d	3.20 $\pm$ 0.037d	2.93 $\pm$ 0.055c	2.77 $\pm$ 0.038d
T ₂	3.49 $\pm$ 0.010a	4.64 $\pm$ 0.019a	4.96 $\pm$ 0.037a	5.43 $\pm$ 0.030a	5.62 $\pm$ 0.035a
T ₃	3.46 $\pm$ 0.012ab	4.51 $\pm$ 0.034b	4.73 $\pm$ 0.025b	5.30 $\pm$ 0.026a	5.43 $\pm$ 0.019b
T ₄	3.42 $\pm$ 0.020b	4.39 $\pm$ 0.019c	4.52 $\pm$ 0.033c	4.90 $\pm$ 0.020b	5.04 $\pm$ 0.023c

Table 5: RBC Count (10⁶/mm³) and WBC Count (10³/mm³) of Tilapia cultured with different colors (red, blue, green and white) of artificial light (Mean $\pm$ SE)

Parameters	Control (T ₀)	T ₁	T ₂	T ₃	T ₄
RBC	1.56 $\pm$ 0.009c	1.46 $\pm$ 0.001d	1.75 $\pm$ 0.005a	1.66 $\pm$ 0.009b	1.56 $\pm$ 0.001c
WBC	26.68 $\pm$ 0.246c	21.76 $\pm$ 0.052d	32.51 $\pm$ 0.071a	28.94 $\pm$ 0.103b	26.99 $\pm$ 0.042c

**Fig 1:** Colouration developed after 60 days

5. Conclusion

The findings of the present study demonstrated that artificial light color significantly influences growth performance, feed utilization, pigmentation and immune response of *Oreochromis niloticus*. Among all treatments, green light (T₃) showed the best overall performance in terms of growth, feed efficiency and survival, while blue light (T₂) was particularly effective in enhancing pigmentation and immune response.

In conclusion, the use of green and blue light can be considered an effective strategy for improving tilapia production in aquaculture systems. Green light is most suitable for maximizing growth performance, whereas blue light plays an important role in improving fish health and pigmentation. These findings suggest that manipulation of light conditions can be a sustainable and efficient approach to enhance aquaculture productivity without the use of chemical additives.

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