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The Growth Response and Digestive Enzyme Activity of Juvenile African Catfish (*Clarias gariepinus*) Exposed to Artificial Light at Night (ALAN) Spectral

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Abstract

Artificial light in various spectral is a relatively new development for modifying the environment of fish. According to research, light has a spectral and species-specific effect on fish.. As a result, the goal of this study was to see how different visible light colors affected *Clarias gariepinus* growth and digestive enzymes. 105 Juvenile fish (body length 10.00 ± 0.55 cm, initial weight 8.67 ± 0.62 g) were randomly exposed in triplicate to the following LEDs: Red (RL), Blue (BL), Green (GL), and Yellow (Y). Total Darkness (TD) and Ambient Light (AL) were used as controls. The fish were exposed for 12 hours overnight for 50 days. At five-day intervals, the fish's body weights were measured with an electric weighing scale (0.01g sensitivity); head length (cm), tail length (cm), and total body length (cm) were measured with a graduated measuring plastic box. The variables listed below were computed: Weight Gain (WG), Daily Weight Gain (DWG), Daily Growth Rate (DGR), Specific Growth Rate (SGR), Percentage Weight Gain (PWG), Food Conversion Ratio (FCR), Length Gain (LG), Daily Length Gain (DLG), Survival Rate (SR) and Condition Factor are some of the metrics used to calculate weight gain. Standard methods were used to measure the activities of digestive enzymes (proteinase and amylase). ANOVA was used to compare the acquired means, and Duncan's multiple range tests were used to further separate the means. Fish reared in YL had significantly higher WG, DWG, DGR, PWG, DLG, LG, and CF levels. SR of fish reared under TD conditions was the lowest, but it was significantly ($P < 0.05$) higher in fish reared under YL and RL conditions. Fish exposed to YL had significantly higher FCR and CF than those exposed to the other light treatments and the Control. The digestive enzyme activities were significantly ($P < 0.05$) reduced during the light treatment. Finally, nighttime artificial light exposure had a significant impact on juvenile catfish growth performance and digestive enzymes, with yellow light eliciting better growth performance.

Keywords: ALAN Spectral, catfish, Growth Performance, digestive enzymes, *Clarias gariepinus.*

1. Introduction

Fish provide some nutritional values for humans such as high levels of protein (Kakoolaki *et al*., 2013), essential amino acids, and easy metabolism by humans of all ages (Ariño *et al*., 2013). Fish is rich in long chain polyunsaturated fatty acids, Omega-3, which are protective against cardiovascular disorders in human (Duran and Talas, 2009). The environment where fish is raised is as vital as most of the body physiology is strongly affected by the environment. Rapid growth in human activity leads to continual expansions in industrialization (Caglar *et al*., 2017) which has an effect on the amount of artificial light entering the aquatic environment and its possible effects on aquatic resources, the effects of which are still mostly unknown.

Light as *zeitgebers*, or time givers, has a large impact on the overall activities of living things. Photoperiod is a critical factor that influences many physiological responses in fish. The use of artificial light in various spectra to modify the environment of fish is a relatively new development. The modification of the fish environment by light has been found to be beneficial to some fishes and detrimental to others. Some fish physiological responses have been reported to be influenced by artificial light. Light, for example, has been shown to influence fish feeding and swimming behavior (Rotllant *et al*., 2003), hormone levels (Boeuf and Le Bail, 1998), basal body metabolism (Almaza'n *et al*., 2004), and skin pigmentation. Photoperiod also influences growth, locomotor activity, metabolic rates, body pigmentation, sexual maturation, and reproduction in fish, according to Biswas *et al*. (2002ab; 2005). Light and background color have been found to impede the detection of feed and the success of feeding of cultured fish in natural settings (Henne and Watanabe, 2003), which in turn influences the overall fitness of the fish. Rainbow trout (*Oncorhynchus mykiss*) larvae grew the fastest when lighting and backdrop color were adjusted to create a contrast in the background for easy identification.

Furthermore, only a few studies looked at the effect of light spectrum on fish; the majority, however, focused on the effects of photoperiod and light intensity. Hybrid catfish and Africa catfish, for example, had the best growth performance in total darkness when raised under different photoperiods (Almazán *et al*., 2004; Mustapha *et al*., 2012; Orina *et al*., 2016). As a result of these findings,

it has been suggested that Africa catfish be raised in complete darkness. However, the physiology of fish, like that of higher vertebrates, may be affected by the light spectrum (Karakatsouli *et al*., 2008).

Furthermore, results from some of the studies revealed that raising fish under different light colors or wavelengths has an impact on both growth performance and the chemical composition of some fish. Elsbaay (2016), for example, reported that Nile tilapia, *Oreochromis niloticus*, exposed to blue light had the best growth performance and that blue light increased amino acid concentration more than other light colors. When exposed to red light, rainbow trout, *O. mykiss*, performed best. Blue light has also been shown to promote the growth and other physiological responses of juvenile Beluga whales, *Huso huso*. Because data on the effect of light spectral on African catfish, *Clarias gariepinus*, is still limited, and it is assumed that they perform better in total darkness (Britz and Pienaar, 2009; Ruchin, 2019), this study aims to add to the existing literature by investigating how LED light wavelengths in the visible spectrum may affect the growth and digestive enzymes of *C. gariepinus*. The Africa cat fish was chosen for this study because it is a popular species in Africa and other parts of the world (Handajani *et al*., 2021).

2. Materials and Methods

2.1. Experimental Site and Housing

The study was carried out in the animal house of the Department of Pure and Applied Zoology, Federal University of Agriculture Abeokuta (FUNAAB), Ogun State.

2.2. Experimental Animal

FUNAAB fish farm provided fish stock (105 in number and 6 weeks old) for the study. The fish were brought to the animal house and acclimatized for seven days in natural conditions.

2.3. Construction of Water Circulation System

For this study, eighteen 30-liter transparent plastic containers were used. 15 of them were wrapped in aluminum foil to prevent external light from passing through and to increase total internal reflection, allowing the radiated light to concentrate within the container. To ensure effective drainage of used water, a water tap was attached to the base of each plastic container. The entire set of plastic containers was linked to a 2000-liter Storex® water tank to ensure a steady supply of clean water to replace the used ones.

2.4. Illumination System

Red, blue, green, and yellow Light Emitting Diode (LED) bulbs were used in three replicates each. The LED bulbs have a power rating of 3 watts and were connected in series via electric flexible cables to a 500-watt solar power system to ensure an uninterrupted power supply during the study period. Each container's inner cover was gummed up with bulbs. Aside from saving energy, these bulbs emit no heat, preventing heat discharge to the fish. Although the exact wavelength of the various light colors used could not be determined, the intensity of the light was measured using a light meter.

2.5. Experimental design

A completely random design was used for distributing fish into various containers. One hundred and five juvenile African catfish (*Clarias gariepinus*) were used; initial weight was 8.67 ± 0.62 g and initial length of 10.00 ±.55 cm. They were distributed randomly among the various light treatments and the control. There were fifteen fish per treatment in a replicate of three and five fish per replicate. The exposure took place at night (7 p.m.–7 a.m.) during the period that the Africa catfish are most active. All the fish experienced ambient light during the day except those in total darkness (Figure 1). The exposure is as indicated: ambient light (12L:12D), blue light (12L:12B), green light (12L:12G), yellow light (12L:12Y), red light (12L:12R), and total darkness (0L:24D). The exposure lasted for 50 days, after which the study was terminated. The ethical guidelines for animal experimentation (regulation CEE 86/609) were strictly followed during the experiment.

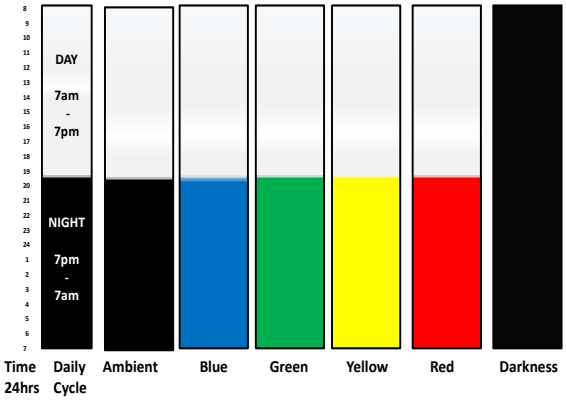

Figure 1. Showing the various light treatment indicating the period of exposure

2.6. Sanitation

Sanitation was done daily by removing remnants of food through the use of rubber hose. Complete changing of used water was usually done every 3 days in order to reduce the ammonia waste.

2.7. The measurement weight and length growth

The body weight and length were measured weekly using an electric weighing scale, and the values were recorded to two decimal places in terms of sensitivity (0.01 g) and one decimal place in terms of meter rule (0.1 cm), respectively. Three catfish were chosen at random from each replicate (for a total of nine fish per treatment), and their weight and length were recorded. The fish was placed in a calibrated transparent container, and its total length was measured.

2.8. Measurement of digestive enzyme

At the end of day 50, three fish from each treatment were randomly selected and sacrificed. The fish tissue was collected, homogenized, and the supernatant was collected for amylase and proteinase analysis.

2.8.1. Measurement of the total amylase (α and β) activity

Sodium acetate buffer of 1/10 M with a pH 5.0 was introduced to 1 ml of the supernatant. The solution obtained was then incubated at 27 °C for 1hr. The action of the enzyme was halted by adding 2 mL of DNSA reagent. The resultant-colored solution gotten was then heated for 5

minutes. The solution was thereafter diluted with distilled water to make up a volume of 10 ml, and this was chilled under running water. The optical density of the solution at 540 nm was compared with a blank. The blank contained 1 ml of identically tested boiling enzyme extract. The amount of reducing sugar produced was calculated. A standard curve for maltose was used to calculate the amount of reducing sugar that was produced (Swain and Dekker, 1966).

2.8.2. Analysis of proteinase activity

The procedure for producing enzyme extracts was the same as that used to produce total amylase extracts, with the exception that the extracting solution was 20 ml of 0.05 M sodium phosphate buffer with a pH 6.0. By adding two milliliters of soluble casein and 0.05 M soluble phosphate buffer with a pH 6.0 to the reaction mixture in order to precipitate unhydrolyzed casein, the Lowry Folin technique was used to measure the proteinase activity in the enzyme extracts (Osborne and Voogt, 1978). The ensuring suspension was centrifuged. A total of 5 ml of 2 % Na_2CO_3, 0.05 ml of 2.7% sodium potassium tartrate, 0.05 ml of 1 % $CuSO_4$, and 3 ml of 0.2 NaOH were poured into 1 ml of the supernatant. Folin-ciocalteu reagent of 0.5 ml was added to the solution formed after 10 minutes, and the mixture was thereafter left for 30 minutes at 30 ^{0}C and shook periodically. At 700 nm, the optical density of the mixture was evaluated in comparison to a blank that contained 1 ml of identically processed boiling enzyme extract. Using a standard curve of different tyrosine concentrations, proteinase activity was estimated (Somkuti and Babel, 1967).

2.9. Data Analysis

Descriptive analysis and linear regression of the gathered data were performed using Excel. ANOVA was used to compare the acquired means, and Duncan's multiple range tests were used to further separate the means. Utilizing SPSS version 22, the analysis was carried out.

3. Results

3.1. The trend of weight increases in African catfish exposed to different light spectral at night

Figure 2 depicts the gradual changes in body weight of African catfish exposed to various light conditions. The regression analysis revealed that the weight change increased significantly ($P < 0.05$) over time, but at a different rate. Fish exposed to YL had the highest R2 value (0.97), followed by fish exposed to red light (0.96), and the lowest was recorded in fish exposed to ambient light, the control condition.

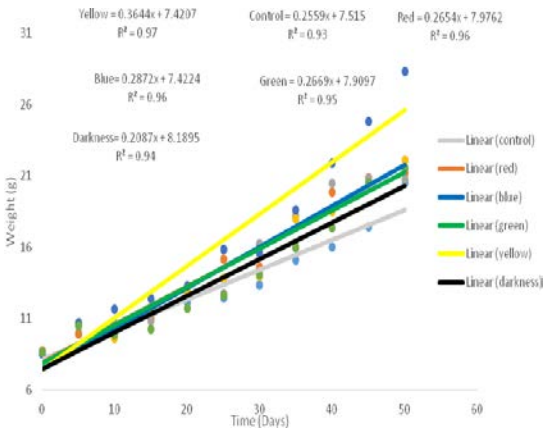

Figure 2. Trend in change in weight of juvenile African catfish exposed to different light spectral over the period of 50 days

3.2. Growth performance (weight basis) of African catfish exposed to different light spectra

Table 1 depicts the effect of various light spectral levels. Fish raised in total darkness gained weight at the same rate ($P > 0.05$) as those raised in ambient light, blue, green, and red light. Yellow light has a significant ($P < 0.05$) effect on final body weight, total body weight, weight gain, daily weight gain, daily growth rate, and percentage weight gain. Food conversion ratio was similar ($P > 0.05$) in catfish exposed to yellow, red, green, and blue lights, as well as darkness, but significantly higher ($P < 0.05$) than in ambient conditions. The fish exposed to yellow light had the highest condition factor (2.220±0.35), but it was not significantly ($P > 0.05$) different from the control (1.490 ± 0.20).

Table 1. Weight Growth indices of African catfish exposed to various light spectral.

LT	Control	Blue	Green	Yellow	Red	Darkness
I W (g)	8.890 ±0.300^a	8.880 ±0.200^a	8.408 ±0.278^a	8.880 ±0.290^a	8.995 ±0.225^a	8.990 ±0.310^a
F W (g)	22.735 ±2.185^a	23.715± 2.955^a	24.345± 2.225^a	33.490± 5.160^b	23.935± 2.735^a	24.380± 2.840^a
T B W (g)	152.670 ±5.200^a	168.680±8.050^a	168.490±8.090^a	196.600±14.760^b	166.320±5.590^a	157.575 ±4.535^a
F C R	0.047 ±0.002^b	0.043 ±0.002^{ab}	0.043 ±0.002^{ab}	0.040 ±0.003^a	0.042 ±0.001^{ab}	0.043 ±0.003^{ab}
W G (g)	14.255 ±2.295^a	15.050 ±2.970^a	15.605±2.155^a	24.955± 5.215^b	15.150±2.710^a	15.890 ±3.030^a
D W G (g)	0.285 ±0.045^a	0.300 ±0.060^a	0.315 ±0.045^a	0.495 ±0.105^b	0.305 ±0.055^a	0.320 ±0.060^a
D G R (g)	3.395 ±0.575^a	3.485 ±0.695^a	3.600 ±0.500^a	5.860 ±1.230^b	3.470 ±0.630^a	3.800 ±0.770^a
S G R (g)	0.850 ±0.110^a	0.850 ±0.120^a	0.890 ±0.090^a	1.155 ±0.175^a	0.845 ±0.105^a	0.900 ±0.130^a
% W G	169.830±28.730^a	174.405±34.745^a	179.950±25.030^a	293.325±61.615^b	173.480±1.510^a	189.885±38.405^a
C F	1.490 ±0.20^a	1.820± 0.23^a	1.805± 0.22^a	2.220±0.35^a	1.580 ±0.56^a	1.630±0.61^a

Note, Means with the same superscript along the column are significantly different ($P < 0.05$)

LT = Light treatment; I W = Initial Weight; F W = Final Weight; T B W = Total Body Weight; F C R = Food Conversion Ratio; W G = Weight gain; D W G = Daily Weight Gain; D G R = Daily Growth Rate; S G R = Specific Growth Rate: C F = Condition Factor

3.3. *Length growth indices of African catfish exposed to different light conditions*

Table 2 displays the length growth indices of African catfish exposed to various light conditions. Exposure to different light conditions had no effect on the FL, LG, LG%, or DLGR ($P > 0.05$). Meanwhile, catfish exposed to yellow light had significantly ($P < 0.05$) longer tail length than those exposed to ambient and red light but were similar ($P > 0.05$) to those exposed to blue, green, and darkness.

Table 2: Length Growth Indices of *Clarias gariepinus*) on Exposure to Different Light Conditions

LT	IL (cm)	FL (cm)	TL (cm)	LG (cm)	% LG (cm)	DLGR (cm)
Ambient	10.6±.7^{ab}	14.3±1.3^{a}	136.1±3.4^{a}	4.0±1.0^{a}	139.0±11.1^{a}	2.7±.4^{a}
Blue	10.7±.4^{ab}	14.3±1.3^{a}	138.2±5.6^{ab}	3.6±1.0^{a}	134.1±9.4^{a}	2.7±.4^{a}
Green	10.6±.7^{ab}	14.7±1.2^{a}	138.6±4.9^{ab}	4.1±1.4^{a}	138.2±14.9^{a}	2.8±.3^{a}
Yellow	10.2±.4^{a}	15.7±2.2^{a}	142.5±6.8^{b}	5.5±2.3^{a}	153.1±23.0^{a}	3.0±.5^{a}
Red	10.5±.5^{ab}	14.7±2.5^{a}	137.1±3.3^{a}	4.2±2.8^{a}	141.1±28.3^{a}	2.8±.6^{a}
Darkness	11.0±.5^{b}	14.8±1.3^{a}	137.6±3.0^{ab}	3.8±1.2^{a}	134.8±10.8^{a}	2.8±.3^{a}

Note, means with different superscript along the column are significantly different ($P < 0.05$)

LT = Light Treatment; IL = Initial length; FL = Final Length; TL = Total length; LG = Length Gain; DLGR = Daily Length Growth Rate

3.4. Percentage Digestive Enzyme of African Catfish Exposed to Different Light spectra.

3.4.1. Amylase of African catfish exposed to different light spectra.

The percentage of digestive enzyme (amylase) in African catfish exposed to different light treatments was significantly ($P < 0.05$) different (Figure 3). The light treatment significantly reduced the activity of amylase as compared with the control (16.3 % ambient and 12.6 % total darkness). The least significant value was reported in the catfish exposed to yellow and green lights (4.3 % and 5.5 %, respectively).

3.4.2. Proteinase percentage of juvenile African catfish exposed to different light spectra.

There was marked ($P < 0.05$) difference in the percentage of proteinase in African catfish exposed to different light treatments. The *C. gariepinus* under yellow and green lights had the least significant ($P < 0.05$) values (1.8 % and 2.0 %, respectively) as compared to the control (ambient 10.5 % and total darkness 7.9 %).

Figure 3. Amylase and proteinase percentage of juvenile African catfish exposed to different light spectral.

3.5. Comparison of the final body weight and digestive enzymes functions of the cat fish exposed to different light wavelengths

Figure 4 depicts a comparison of final body weight and digestive enzyme activity. Except for red light, there is an inverse relationship between digestive enzymes and body weight that is proportional to the wavelength of the light. The enzymes' activities decreased as the wavelength increased. The growth indicator, on the other hand, was found to increase as the wavelength of the light increased.

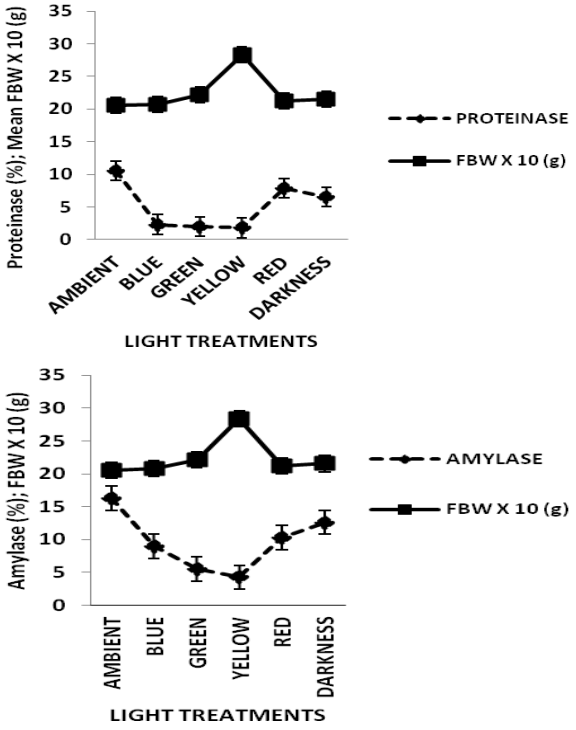

Figure 4. Comparison between the final body weights (FBW) and the digestive enzymes (Proteinase A, and Amylase B) functions of catfish exposed to light of various wavelengths

3.6. Survival rate of African catfish (Clarias gariepinus) exposed to different light condition.

In terms Survival rate, result showed that fish reared under yellow and red lights had the highest survival rate of 80.95 %, while those of blue, green and ambient condition had 61.90 %. Those under dark condition gave the lowest survival rate (52.38 %). This implies that the highest percentage of death rate was recorded in those under dark condition, while those under yellow and light spectral survived best (Figure 5).

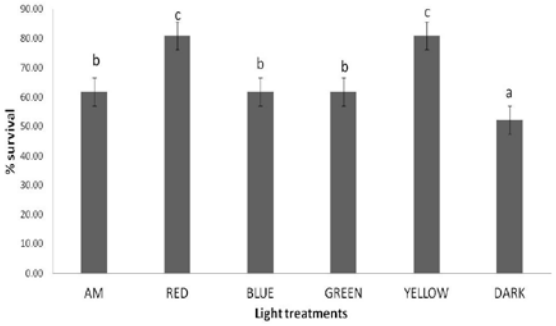

Figure 5 . Survival rate of juvenile African catfish (*Clarias gariepinus*) exposed to different light spectral for the period of 50 days.
Mean with dissimilar superscript are significantly different ($p < 0.05$)

4. Discussion

This study investigated how changing light wavelengths affected the development and digestive enzyme activity of *C. gariepinus*. The influence of photoperiod on *C. gariepinus* physiological processes has been studied. According to reports, the various life stages of *C. gariepinus* thrive best in absolute darkness as opposed to ambient light. Throughout this investigation, the same correlation was detected between ambient light and darkness.

The growth indicators demonstrated in this study that *C. gariepinus* responds favorably to yellow light. The outcome is congruent with what has been seen elsewhere. Shittu (2015) found the same phenomenon in *C. gariepinus* exposed to yellow light, with the exception that he utilized fluorescent bulbs instead of LEDs in his investigation. In accordance with this study, Sallehudin *et al*. (2017a) demonstrated that when African catfish were exposed to various light hues for fourteen days, yellow light promoted the greatest growth. Some studies on the impact of light hue on the growth performance of various fish have produced contradictory findings. For instance, the growth performance of Nile tilapia, *Oreochromis niloticus*, was greatest when exposed to red light (Lopez-Betancur *et al*., 2020), although Elsbaay (2013; 2016) indicated that blue light was more effective. Nasir and Farmer (2017) found the optimum growth performance for Common carp, *Cyprinus carpio*, under red light, and Ruchin *et al*. (2002) under green light. In addition, Dadfar *et al*. (2017) showed that the weight parameters of rainbow trout, *O. mykiss*, were greater under yellow and white lighting as opposed to Karakatsouli *et al*. (2008), who stated that *O. mykiss* performed best under red light. The juvenile Beluga, *Huso huso*, performed best under blue light (Banan *et al*., 2010), while Snakehead performed best when exposed to green light.

It is still unclear how colored light affects fish in general and Catfish specifically in terms of growth performance. In order to determine whether there is a direct relationship between the color of the light and the activity of the enzymes, this study examined the effect of colored light on the function of the digestive system. How well food is absorbed during digestion has been shown to be significantly influenced by the actions of the digestive enzymes (Sanchez-Muros *et al*., 2013). According to Fereshteh *et al*. (2016), pancreatic enzyme activities increase along with photoperiod, which improves the growth performance of juvenile rainbow trout. This implied that the light diet might have an impact on how well the digestive enzymes work. According to this study, enzyme activity decreased when exposed to light and then increased as the light's wavelength increased. The enzyme activity and growth efficiency under yellow light had a complete inverse relationship. One would think that since yellow light exposure produced the best growth results, the enzyme's activity would be at its peak, but the opposite was true. If the enzymes' activity had been found to be higher in the presence of yellow light, one might have hypothesized that the upregulation of the enzymes would result in greater digestion, which would then produce more energy and promote greater growth. However, since the enzyme's activity was now markedly diminished by yellow light, the growth performance could no longer be directly linked to food digestion, which would increase the energy available. The growth response observed under the yellow light must be caused by something other than food since the activity of the enzymes does not directly correlate with growth, and this other cause is still unknown.

In addition, Sallehudin *et al.* (2017b) reported that Africa catfish exposed to yellow light showed less aggression as compared to other light colors. Since the same authors had reported better growth performance under yellow light in their earlier work (Sallehudin *et al.*, 2017a), then the assumption one can make is that the energetic cost on aggressive behavior might have been channeled on building of the muscles and the skeletal system hence, the better growth performance observed. There is a need for more investigations to know the mechanism by which light colors help enhance growth performance in fish.

5. Conclusion

Weight and length of juvenile African catfish (*C. gariepinus*) were affected by monochromatic lights. Yellow light improved daily growth rate, weight gain, and length gain in juvenile *C. gariepinus*. Yellow and red lights increase the survival rate of *C. gariepinus*. Blue, green, and yellow lights reduced the activities of amylase and protease.

6. Recommendation

We want to recommend that the actual wavelength of yellow light that will elicit the best response be examined. Also, the proximate analysis of the fish should be evaluated so as to know the part of the fish's nutrition that may be affected due to the exposure. It will also be of interest to evaluate the amino acid and lipid profiles of the fish after the exposure so as to ascertain the nutritional value of the fish after the exposure.

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