UNIVERSITI PUTRA MALAYSIA

EFFECT OF RED AND BLUE LIGHTS ON THE STRESS RESPONSE AND GROWTH PERFORMANCE OF JUVENILE RED TILAPIA (*Oreochromis sp.*)

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Effect of Red and Blue Lights on the Stress Response and Growth Performance of Juvenile Red Tilapia (*Oreochromis sp.*)

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A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfillment of the requirement for the
DEGREE DOCTOR OF VETERINARY MEDICINE
Universiti Putra Malaysia
Serdang, Selangor Darul Ehsan.

MARCH 2016

CERTIFICATION

It is hereby certified that we have read this project paper entitled "Effect of red and blue lights on the stress response and growth performance of juvenile red tilapia (*Oreochromis sp.*)", by Cheah Siew Siew and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999- Project.

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DEDICATION

To
the love in life,
the peaceful mind,
and the beauty of every single soul.

AKNOWLEDGEMENTS

First thanks to my most respectful supervisor, Prof. Shariff for all he have taught: about life, about dream, about imagination, about power of thinking and about the humbleness he always bring along with. It is such a good luck I have to be supervised by you in this journey.

Next thanks to Dr.Sanjoy, my always smiling co-supervisor, who also always be there and gave me a hand whenever I need it. Nothing much that I could do to repay your kindness, but I guess to do the best in this project is the best way to show my appreciation to you.

Sincere thanks to Miss Kumari and Dr. Diyana, for their support and guide throughout the whole project. Love to listen the stories both of you have experienced. I would always pray for you. A bright and lovely future shall be waiting there for us and what we need to do is just go step by step.

Appreciate the help from Greg, who made the light fixing possible in short period of time. You are really amazing and proficient in something new! Thanks Pearl for being so helpful and take care of me like a sister. You shall be a pretty and intelligent Doctor soon!

Thanks you to staff in hematology and clinical pathology laboratory: Mr. Halmi, Mr. Abdullah Misron, Mr. Arman Addelan, Miss Darulmuqaamah and Miss Liyana. Their professional in hematology and clinical pathology field should be respected and appreciated by all of us.

Thousand thanks to all lecturers and staffs in Faculty of Veterinary Medicine. Each of you have been there to lead me step by step to the gateway of graduating successfully.

Millions thanks to school teachers, who never gave up on little children like me and brought them hope along the way. They are the one who build the basement for me.

Billion thanks to family, and to friends. Their endless supports have been warming my coolest day and open up the door for sunlight to shine in. You all are the beautiful gifts I have in this life.

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LIST OF ABBREVIATIONS

%	percent
<	less than
ºC	degree Celsius
α	alpha
$\mu mol/m^2/sec$	micromole per metre square per second
Cl	Calcium
cm	centimeter
fL	femtoliter
G	Gauge
g	gram
hsps	heat-shock proteins
K	Potassium
L	Liter
LED	Light Emitting Diode
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
mg	milligram
mL	milliliter
mmol	millimole
MS222	Tricaine methanesulfonate
n	number
Na	Sodium
nm	nanometer
P	Probability
PCV	Packed Cell Volume
pH	potential of Hydrogen
ppt	parts per thousand
ppm	parts per million
RBC	Red Blood Cell
RCF	Relative Centrifuge Force
SEM	Standard Error of the Mean
sp.	species
WBC	White Blood Cell

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

KESAN LAMPU MERAH DAN BIRU KEPADA TINDAK BALAS TEKANAN DAN PRESTASI PERTUMBUHAN JUVANA TILAPIA MERAH (*Oreochromis sp.*)

Oleh

Cheah Siew Siew

2016

Penyelia: Professor Dato' Dr. Mohamed Shariff Mohamed Din

Penyelia bersama: Dr. Sanjoy Banerjee

Warna cahaya didapati mempunyai kesan ke atas tindak balas tekanan dan prestasi pertumbuhan di beberapa spesies ikan. Kajian ini menilai kesan cahaya dengan gelombang terpanjang (merah) dan gelombang terpendek (biru) pada tindak balas tekanan dan prestasi pertumbuhan juvana tilapia merah sepanjang 23 hari eksperimen. Cahaya merah dan biru ditetapkan sebagai kumpulan rawatan manakala cahaya putih ditetapkan sebagai kawalan dengan lampu dipasangkan di atas tangki yang ditutup dengan plastic yang berwarna hitam dan legap. Darah diambil daripada ikan yang dipilih secara rawak (n = 45) pada hari 0, 1, 3, 8, 15, dan 23 untuk menilai parameter hematologi (kiraan sel darah merah, hemoglobin, isi padu sel padat, min isi padu korpusel, min kepekatan hemoglobin korpusel, kiraan sel darah putih, trombosit, protein plasma) dan biokimia plasma (trigliserida , glukosa, jumlah protein, albumin, globulin, dan ions- utama Na, K, Cl). Panjang badan dan berat badan juga diukur pada hari 0 dan hari 23 eksperimen untuk menilai purata ketambahan panjang badan, pertambahan berat badan, kadar pertumbuhan spesifik dan kadar penukaran makanan. Hasilnya menunjukkan bahawa tidak terdapat perbezaan yang signifikan ke atas tindak balas tekanan antara cahaya merah, biru dan putih. Cahaya biru telah menunjukkan pertambahan kepanjangan badan yang signifikan (P = 0.002) berbanding dengan cahaya putih walaupun parameter pertumbuhan lain seperti pertambahan berat badan, kadar pertumbuhan spesifik dan kadar penukaran makanan) adalah tidak signifikan antara rawatan tiga cahaya.

Kata kunci: Warna cahaya, merah, biru, putih, parameter hematologi, biokimia plasma, tindak balas tekanan, prestasi pertumbuhan, juvana red tilapia.

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Project

EFFECT OF RED AND BLUE LIGHTS ON THE STRESS RESPONSE AND GROWTH PERFORMANCE OF JUVENILE RED TILAPIA (*Oreochromis sp.*)

By

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2016

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Colour of light is found to have effect on stress response and growth performance of several species of fish. This study evaluated the effect of visible light with longest wavelength (red) and shortest wavelength (blue) on the stress response and growth performance of juvenile red tilapia throughout 23 days of experiment. Red and blue lights as treatment groups while white light as control group were fixed underneath the tank lid and covered with opaque black colour plastic. Blood was withdrawn from fish chosen randomly (n=45) on day 0, 1, 3, 8, 15, and 23 to evaluate the hematological parameters (RBC count, hemoglobin, PCV, MCV, MCHC, WBC count, thrombocytes and plasma protein) and plasma biochemistry (triglycerides, glucose, total protein, albumin, globulin, and major ions- Na, K, Cl). Body length and weight were also measured on day 0 and day 23 of experiment to evaluate the average length gain, body weight gain, specific growth rate and feed conversion rate. The result showed that there was no significant difference on stress response among the red, blue and white lights. Blue light showed significant length gains (P=0.002) as compared to white light although the other growth parameters (body weight gain, specific growth rate and feed conversion rate) were not significant between treatment groups (red and blue lights) and control group (white light).

Key words: Colour of light, red, blue, white, hematological parameters, plasma biochemistry, stress response, growth performance, juvenile red tilapia.

Introduction

Artificial lighting is one of the environmental factors that have been concerned in indoor intensive rearing of aquaculture. Research on lighting photoperiod, intensity and spectrum have shown some physiological changes on fish growth (Boeuf and Le Bail, 1999; Tamazouzt et al., 2000; Villamizar et.al., 2014), behaviour (Marchesan et al., 2005; Owen et al. , 2010) and stress response (Karakatsouli et al., 2012; Migaud et al., 2007; Owen et al, 2010). In recent years, there is increased interest to investigate the most suitable lighting spectrum in order to encourage optimum production of fish species. Red light, as the longest wavelength of visible light was found to stimulate feeding motivation in Nile tilapia (Volpato et al., 2013). Providing that stressors are kept to a minimum, red light also favoured length gain in rainbow trout (Karakatsouli et al., 2008) and yellow perch (Head and Malison, 2000). However, in a recent study on the effect of light spectrum in Atlantic cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*), larvae of both species showed significantly enhanced growth under treatments of blue and green lights as compared to red light (Sierra et al., 2016).While for another study done by Karakatsouli et al. (2008), blue light was found to favour acute stress response in rainbow trout. These studies have suggested that effect of light spectrum on physiological changes could be species specific. Hence, a proper understanding of the fish reaction towards different light spectrum should be done in more fish species, especially those with commercial interest, in order to provide optimum environment factors in aquaculture sector.

In Malaysia, tilapia has grown to be one of the important freshwater fish by contributing 33% of freshwater aquaculture production in 2013 (Mazuki, 2015). With the growing interest on relationship of light spectrum and commercial fish species, this study aims to investigate the effect of lighting spectrum towards physiological changes of juvenile red tilapia, in terms of stress response and growth performance.

Activation of stress response usually indicates that the animal is responding to a challenge (Pottinger, 2008) by a suite of endocrine events (Johnson et al, 1992), followed by suite of cardio-respiratory function accompanied by mobilization of carbohydrates and lipid reserves (Pottinger, 2008), whose ultimate purpose is to protect or reestablish homeostasis.

In this study, the physical stressor: red light and blue light were exerted to the juvenile red tilapia for 23 days. At the end of experiment, blood was withdrawn to evaluate stress response in terms of hematological parameters and plasma biochemistry, especially plasma glucose. Total length and weight of each fish was also taken to evaluate the growth performance throughout the study period. Thus the objectives of this study were to:

1. Compare the effect of red and blue lights on stress response in terms of hematological parameters and plasma biochemistry of juvenile red tilapia.
2. Compare the effect of red and blue lights on growth performance of juvenile red tilapia.

Literature Review

2.1 Red and blue lights in water

Light passed through water undergo reflection, refraction and absorptions, leading to wide variation in light transmission and spectral irradiance at different depth of water (Partridge, 1990). Among the colours of visible light, blue light (436nm wavelength) has highest photon energy and penetrate deepest in water; red light (689nm wavelength) has lowest photon energy and being absorbed the most (Jerlov, 1968). The presence of broad range of spectral sensitivity in fish proved the ability of fish to see different colours under water (Douglas and Hawryshyn, 1990). Ekstrzm and Meissl (1997) suggested that light spectrum is detected by the eye and pineal gland in fish. Although recent studies have proven that environmental colours are important on physiology and behavior (Karakatsouli et al., 2008, Barcellos et al., 2006, Owen et al., 2010 and Imanpoor et al., 2011), the mechanism leading to these physiological changes are still unclear. Sensitivity of a fish's retina could have been tuned to the wavelength range of environment in which it lives (Partridge and Cummings, 1999; Hornsby et al., 2013), hence a deviation of wavelength in aquatic environment such as red light could have disturbed the normal living condition, that may influence physiological changes on fish. While when a stressed fish confined under blue light (the nearest colour similar to aquatic environment), it shown to decrease stress level significantly, suggesting the colour improves fish well-being so that mild stressors are better tolerated (Volapato and Barreto, 2001; Maia and Volpato, 2013).

2.2 Overview of stress response in fish

The scheme of stress induced neuroactivation in fish is similar to other vertebrates (Tort and Teles, 2011). When a stressor is detected, stimulus is first processed in central nervous system, then activate the hypothalamus-pituitary-adrenal axis (Randall and Perry, 1992). Two stress hormones, cortisol and catecholamine (adrenaline and epinephrine) are released into the bloodstream which represents the primary stress response of fish (Pottinger, 2008). Reid et al. (1998) and Mommsen et al. (1999) have suggested that the elevated level of stress hormones modulate cardiovascular and respiratory functions, as well as the metabolic effect. These physiological adjustments such as in metabolism, respiration, acid-base status, hydromineral balance, immune function and cellular responses represents the secondary stress response. The consequences from the primary and secondary responses are seen as changes in the whole animal performance, such as in growth, disease resistance and behavior, which is known as tertiary stress response (Barton, 2002).

2.2.1 Stress response in terms of hematological parameters

Hematological parameters can be the stress indicators of fish by monitoring the changes in hemoglobin, number of red blood cell and hematocrit values (Schreck et al., 1997). Hematological disorders such as polycythemia and erythrocyte swelling can be observed in stressed fish resulting from release of catecholamine (Clauss et al., 2008). In chronic environmental or physical stress, studies found that there were increased blood hemoglobin and PCV, probably due to high energy demand and high metabolic rates (Mirea et al., 2013; Placinta et al., 2014; Latif et al., 2015). Same as in mammals, stressed fish show decrease leucocyte number (leukopenia) as indication of stress response (Maule and Schreck, 1990; Ainsworth, 1991; Schreck et al., 1997). Besides, Chen at al., (2002) and Law et al., (2001) found that stress modulated the changes of catecholamine and cortisol and further depressed phagocytic activity and antibody levels in fish.

2.2.2. Stress response in terms of plasma biochemistry.

Fish can react towards stressor by showing measurable changes in blood glucose, lactate or lactic acid, and major ions for example chloride, sodium, and potassium (Barton, 2002). The release of cortisol is known to elevate glucose production through gluconeogenesis and glycogenolysis pathways in order to provide sufficient energy for fish to cope with stress (Iwama et al., 1999). However, it is recommended that glucose should be measured over time instead of a single reading in case of stress study (McDonald and Milligan, 1997). Besides, total protein made up of albumin and globulin can have clinical relevance in fish by showing increase or decrease in amount after a pathological or stress situations (Peres et al., 2015). Immunosuppressive effect of stress can then be well characterized through total protein count. In addition, the release of adrenaline as result of primary stress response has led to the osmotic disturbance which increased ions (Na, Cl , K) when compared to non- stressful condition (McDonald and Milligan, 1997).

2.3. Growth performance in fish

Both environmental variables (for example water quality, photoperiod and temperature) and hormones (catabolic and anabolic) can influence fish growth at bioenergetics level (Van Weerd and Koman, 1998). In few studies, decline in growth hormone were correlated with increase in plasma cortisol level. It has been proven that increased glucocorticoids level in mammal can inhibit growth hormone via somatostatin (Giustina and Wehrenberg 1992). The same mechanism could have happen in fish as somatostin was found to reduce growth hormone binding in trout liver (Very and Sheridan, 2007). Besides, increase glycogen mobilization to meet the energy demand imposed by the stressor could have reduced the growth potential (Vijayan and Moon, 1992). The performance of growth can be assessed through growth hormone level (Perez-Sanchez and Le Bail, 1999). Length and weight relationship is also important to analyse growth (Patnaik et al., 2014).

Material and Method

3.1 Experimental design

Four hundred and fifty live specimens of 60 days old juvenile red tilapia (*Oreochromis sp.*) were acclimatized in nine transparent glass tank (n=50 for each tank) for 11 days. The volume capacity for each tank was 175.69 L, with length x height x width =121 x 33x 44 cm. After acclimatization, fish were subjected to different colours of LED light: white (as control), blue and red colours (as treatments) for 23 days, by installing the LED lights underneath the cover lid in each tank (three tanks for each colour). All tank sides were covered with black opaque plastic in order to avoid complication due to room lighting. Continuous aeration was given through a biological filter for each tank. Fish were fed twice daily, in the morning and evening, with commercial pellet (32% crude protein) at a rate of 3% of body mass. Water quality parameters were maintained as follow: salinity, 0.29 ± 0.18 ppt; temperature, 29.97 ± 1.25ºC; dissolved oxygen, 4.6 ±1.1 mg/L; pH, 6.88 ± 0.3. The light intensity for all tanks were adjusted to 50 μmol/m^2/sec measured from water surface while the light photoperiod was maintained at 12 hours light: 12 hours dark daily.

3.2 Sampling and analytical methods

Blood and measurement of length and weight were taken from fish one day before different light treatments and day 1, 3, 8, 15, 23 of different light treatments. Every time prior to fish sampling, fish were starved for 24 hours then anaesthetized with 200ppm of MS222. Five fish from each tank were chosen randomly then length and weight for each fish were measured using Vernier caliper and weighing scale. Blood was withdrawn from caudal vein puncture, using 25G hypodermic needle and 1mL syringe then stored in lithium heparinized vacutainer. Next, the red blood cell, hemoglobin value, total white blood cell and thrombocytes were counted using Abbott Diagnostic CELL-DYN® 3700 machine. PCV was evaluated after centrifuge (12879.36 RCF for 5 minutes) while plasma protein value was measured through refractometer. The remaining blood was again centrifuged (2360 RCF for 5 minutes) and obtained plasma was used for the determination of glucose, triglycerides, total protein, albumin and major ions (potassium, sodium, calcium) using Hitachi 902 Automatic Analyzer with Roche Hitachi reagent.

3.2 Statistical analysis

The growth performance parameters (average length gain, body weight gain, specific growth rate and feed conversion rate) before and after 23 days of different light treatments was calculated and compared in bar chart (Figures 4.14, 4.15, 4.16 and 4.17). The hematological parameters and plasma biochemistry for each colour of different light treatments throughout the experiment are shown in line graphs (Figures 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 4.10, 4.11, 4.12, and 4.13). All data were then analysed using one way analysis of variance (ANOVA) with treatment as independent factor. The average values were compared at significant level $\alpha=0.05$. Where P values were significant ($P<0.05$), multiplication comparisons were carried out using the Duncan test. Wherever necessary, data was analysed with Welch's test in order to obtain homogeneity of variance. All data are presented as mean values ±SEM.

Result

4.1 Hematological parameters and plasma biochemistry

There were no significant differences of the hematological parameters (RBC count, hemoglobin, PCV, MCV, MCHC, WBC count, thrombocytes and plasma protein) and plasma biochemistry (triglycerides, glucose, total protein, albumin, globulin, and major ions- Na, K, Cl) among fish treated with white (control), blue and red (treatments) lights on day 1, 3, 8, 25 and 23 (Tables 4.1, 4.2, 4.3, 4.4 and 4.5).

Table 4.1: Hematological parameters and plasma biochemistry of juvenile red tilapia after 1 day of different light treatments.

Light treatments (number of fish)	White (n=15)	Red (n=15)	Blue (n=15)	Sig.	
RBC (x10^{12}/L)	2.36±0.15	2.37±0.31	2.54±0.12	0.789	NS
Hb (g/L)	114.33±1.45	101.87±4.48	99.33±5.79	0.068	NS
PCV (L/L)	0.29±0.02	0.33	0.32±0.01	0.161	NS
MCV (fL)	124.67±5.24	144.67±21.70	128±10.26	0.593	NS
MCHC (g/L)	357±11.15	354.67±4.81	342.67±20.33	0.740	NS
WBC (x 10^9/L)	1.77±0.48	1.20±0.49	1.16±0.32	0.582	NS
Thrombocyte (x 10^9/L)	6.01±3.50	8.15±3.03	2.57±0.31	0.390	NS
Plasma Protein (g/L)	27.67±0.33	31.33±2.60	32±2.31	0.331	NS
Triglycerides (mmol/L)	1.81±0.39	1.92±0.88	2.12±0.83	0.956	NS
Total protein (g/L)	28.73±0.41	30.30±1.99	31.67±1.00	0.347	NS
Albumin (g/L)	8.83±0.58	8.47±0.68	8.83±0.50	0.593	NS
Globulin (g/L)	19.90±0.79	21.83±1.31	22.83±1.32	0.271	NS
A:G	0.45±0.05	0.39±0.01	0.39±0.04	0.476	NS
Glucose (mmol/L)	3.27±0.20	3.30±0.10	3.10±0.15	0.650	NS
Na (mmol/L)	162.13±1.80	165.40±1.45	164.90±1.47	0.355	NS
K (mmol/L)	1.90±0.86	1.47±0.15	1.53±0.27	0.828	NS
Cl (mmol/L)	138.40±0.60	139.30±0.85	139.87±0.98	0.492	NS

*The table shows mean±SEM of three replicate tanks per treatment.

*P value <0.05 indicates significant difference.

*NS= Not significant.

Table 4.2: Hematological parameters and plasma biochemistry of juvenile red tilapia after 3 days of different light treatments.

Light treatments (number of fish)	White (n=15)	Red (n=15)	Blue (n=15)	Sig.	
RBC ($x10^{12}/L$)	2.31±0.19	2.29±0.20	2.27±0.27	0.993	NS
Hb (g/L)	101.93±9.35	100.63±7.37	111.67±6.12	0.574	NS
PCV (L/L)	0.29±0.02	0.33	0.32±0.01	0.680	NS
MCV (fL)	122.33±2.73	119±1.53	136.67±8.29	0.105	NS
MCHC (g/L)	361±9.24	370±12.17	365.33±7.84	0.819	NS
WBC ($x\ 10^{9}/L$)	1.44±0.07	1.37±0.57	1.84±0.47	0.713	NS
Thrombocyte ($x\ 10^{9}/L$)	5.62±0.99	10.18±5.32	4.36±2.23	0.483	NS
Plasma Protein (g/L)	27±2.08	27.33±1.45	28.33±2.33	0.888	NS
Triglycerides (mmol/L)	1.16±0.16	1.36±0.19	1.43±0.42	0.788	NS
Total protein (g/L)	22.23±4.47	26.63±2.48	29.03±1.03	0.337	NS
Albumin (g/L)	9.70±0.57	9.73±0.58	10.17±0.20	0.758	NS
Globulin (g/L)	12.53±4.33	16.90±2.17	18.87±0.87	0.34	NS
A:G	1.20±0.61	0.59±0.08	0.54±0.02	0.407	NS
Glucose (mmol/L)	3.30±0.31	2.93±0.07	2.73±0.07	0.169	NS
Na (mmol/L)	167.10±2.48	164.67±0.55	168.67±1.24	0.291	NS
K (mmol/L)	3.83±0.71	4.20±1.26	1.60±0.31	0.143	NS
Cl (mmol/L)	148.40±3.04	146.03±1.43	145.63±0.61	0.591	NS

*The table shows mean±SEM of three replicate tanks per treatment.

*P value <0.05 indicates significant difference.

*NS= Not significant

Table 4.3: Hematological parameters and plasma biochemistry of juvenile red tilapia after 8 days of different light treatments.

Light treatments (number of fish)	White (n=15)	Red (n=15)	Blue (n=15)	Sig.	
RBC ($x10^{12}/L$)	2.51±0.02	2.50±0.10	2.23±0.08	0.061	NS
Hb (g/L)	101.07±3.73	112.33±5.24	102±3.61	0.197	NS
PCV (L/L)	0.29±0.02	0.33	0.32±0.01	0.235	NS
MCV (fL)	132.67±6.39	127±3.51	128.67±3.93	0.705	NS
MCHC (g/L)	345.33±4.33	354.33±10.27	356±2.08	0.505	NS
WBC ($x\ 10^9/L$)	1.75±0.15	1.27±0.05	1.16±0.29	0.145	NS
Thrombocyte ($x\ 10^9/L$)	13.13±5.53	8.64±2.91	4.58±2.36	0.357	NS
Plasma Protein (g/L)	26.67±2.67	27.67±1.20	26±2.31	0.862	NS
Triglycerides (mmol/L)	1.45±0.36	1.58±0.30	1.69±0.77	0.904	NS
Total protein (g/L)	22.57±3.09	26.40±5.01	19.93±3.18	0.529	NS
Albumin (g/L)	8.37±0.63	9.17±0.23	8.43±0.76	0.881	NS
Globulin (g/L)	14.20±2.64	17.23±4.92	11.50±2.79	0.563	NS
A:G	0.63±0.11	0.68±0.26	0.80±0.14	0.799	NS
Glucose (mmol/L)	3.63±0.20	3.17±0.29	3.30±0.17	0.388	NS
Na (mmol/L)	160.13±1.41	162.53±2.04	161.27±0.57	0.291	NS
K (mmol/L)	3.37±1.11	2.40±0.21	2.57±0.39	0.596	NS
Cl (mmol/L)	140.60±1.39	140±2.05	141.83±0.99	0.706	NS

*The table shows mean±SEM of three replicate tanks per treatment.

*P value <0.05 indicates significant difference.

*NS= Not significant.

Table 4.4: Hematological parameters and plasma biochemistry of juvenile red tilapia after 15 days of different light treatments.

Light treatments (number of fish)	White (n=15)	Red (n=15)	Blue (n=15)	Sig.	
RBC (x10^{12}/L)	2.12±0.29	2.42±0.09	2.05±0.16	0.423	NS
Hb (g/L)	105.9±10.07	108.27±5.61	93.47±6.42	0.395	NS
PCV (L/L)	0.32±0.04	0.33±0.01	0.28±0.02	0.341	NS
MCV (fL)	152±6.03	138±3.06	139±3	0.106	NS
MCHC (g/L)	332±4.73	324.67±10.97	329.67±4.06	0.776	NS
WBC (x 10^9/L)	1.49±0.17	1.48±0.11	1.08±0.14	0.148	NS
Thrombocyte (x 10^9/L)	3.92±1.18	5.10±1.07	2.42±1.38	0.359	NS
Plasma Protein (g/L)	30±3	35.67±1.67	27.67±1.20	0.084	NS
Triglycerides (mmol/L)	1.79±0.34	1.99±0.42	1.30±0.12	0.351	NS
Total protein (g/L)	32.10±2.36	36.40±0.95	30.07±0.58	0.061	NS
Albumin (g/L)	10.67±0.94	12.70±0.45	11.50±0.52	0.281	NS
Globulin (g/L)	21.43±1.43	23.70±1.19	18.57±0.27	0.043	NS
A:G	0.50±0.01	0.54±0.04	0.62±0.03	0.077	NS
Glucose (mmol/L)	3.40±0.21	3.57±0.47	3.33±0.30	0.885	NS
Na (mmol/L)	164±1.65	166.23±1.10	165.47±0.65	0.46	NS
K (mmol/L)	1.60±0.40	0.93±0.09	1.07±0.22	0.255	NS
Cl (mmol/L)	139.13±2.10	138.37±0.78	140.30±0.96	0.641	NS

*The table shows mean±SEM of three replicate tanks per treatment.

*P value <0.05 indicates significant difference.

*NS= Not significant.

Table 4.5: Hematological parameters and plasma biochemistry of juvenile red tilapia after 23 days of different light treatments.

Light treatments	White (n=15)	Red (n=15)	Blue (n=15)	Sig.	

(number of fish)					
RBC (x10^{12}/L)	2.18±0.13	2.18±0.12	2.46±0.02	0.162	NS
Hb (g/L)	105±2.52	105.67±3.28	111.67±3.53	0.324	NS
PCV (L/L)	0.33±0.01	0.33±0.01	0.34±0.01	0.665	NS
MCV (fL)	154±11	152±5.03	137.67±1.33	0.276	NS
MCHC (g/L)	315.33±7.69	320.33±2.60	328.33±7.97	0.423	NS
WBC (x 10^{9}/L)	1.29±0.32	1.14±0.11	1.25±0.09	0.865	NS
Thrombocyte (x 10^{9}/L)	7.57±1.94	3.22±1.42	4±1.30	0.196	NS
Plasma Protein (g/L)	33.33±4.67	34.67±1.76	33.67±1.20	0.947	NS
Triglycerides (mmol/L)	2.13±0.86	2.72±0.54	1.91±0.35	0.654	NS
Total protein (g/L)	33.60±3.35	36.80±1.96	31.90±0.35	0.359	NS
Albumin (g/L)	10.60±1.27	11.30±0.47	10.30±0.45	0.694	NS
Globulin (g/L)	23±2.11	25.50±1.56	21.60±0.67	0.277	NS
A:G	0.46±0.02	0.44±0.02	0.48±0.04	0.624	NS
Glucose (mmol/L)	2.93±0.38	3.00±0.31	3.23±0.46	0.851	NS
Na (mmol/L)	161.90±1.51	166.40±1.75	161.70±0.25	0.082	NS
K (mmol/L)	1.83±0.49	1.50±0.25	1.43±0.81	0.639	NS
Cl (mmol/L)	138.57±2.67	141.20±1.95	137.57±0.70	0.446	NS

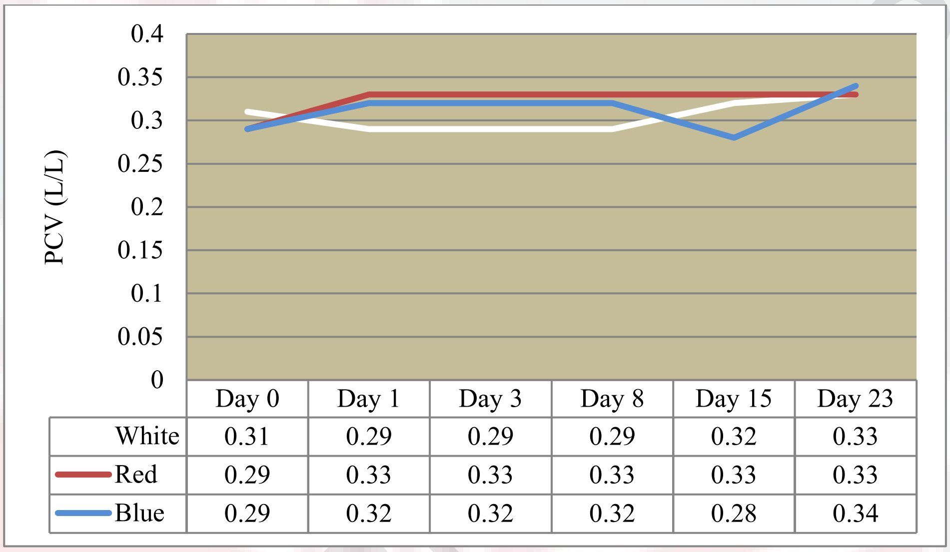
*The table shows mean±SEM of three replicate tanks per treatment.

*P value <0.05 indicates significant difference.

*NS= Not significant.

The difference in packed cell volume among the light treatments was not significant even though red and blue lights appeared to have high PCV (0.33L/L and 0.32 L/L respectively) compared to white light (PCV= 0.29L/L) on day1, 3 and 8 (Figure 4.1).

Figure 4.1: Effect of different light treatments of fish and their resulting packed cell value throughout 23 days of study.



No significant different for red blood cell, hemoglobin, white blood cell count and plasma protein value was observed among the light treatments (Table 4.1; Figures 4.2, 4.3, 4.4 and 4.5) although fish with blue light appeared to have high RBC count (2.54 x 10^9/L) on day 1, high hemoglobin (111.67g/L) and WBC count (1.84 x 10^9/L) on day 3 and red light showed high RBC count (2.50 x 10^9/L), hemoglobin (112.33 g/L) on day 8 and 15, high plasma protein (35.67 g/L) on day 15. White light appeared to have high mean value of WBC count on day 3 (1.77 x 10^9/L) and day 8 (1.75x 10^9/L), but the difference with other treatments was also not significant.

Figure 4.2: Effect of different light treatments of fish and their resulting red blood cell count throughout 23 days of study.

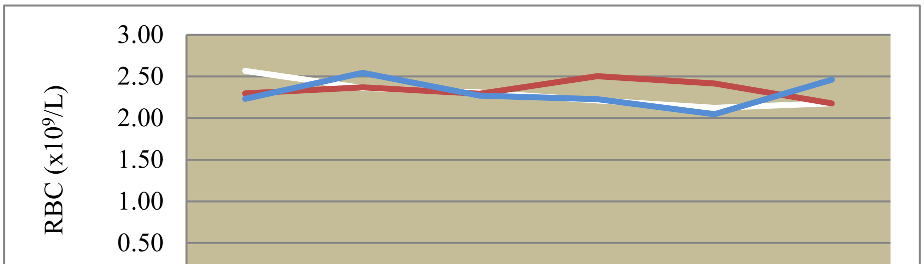
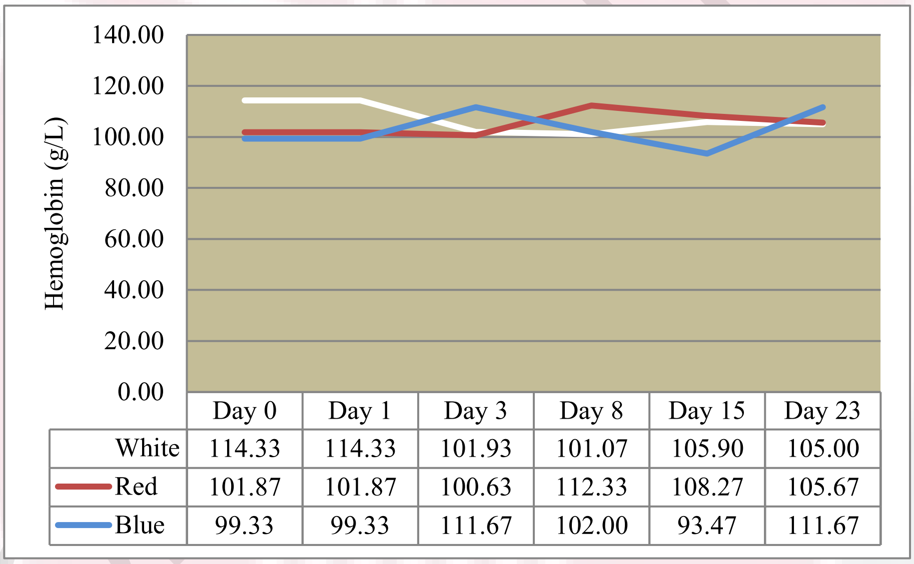
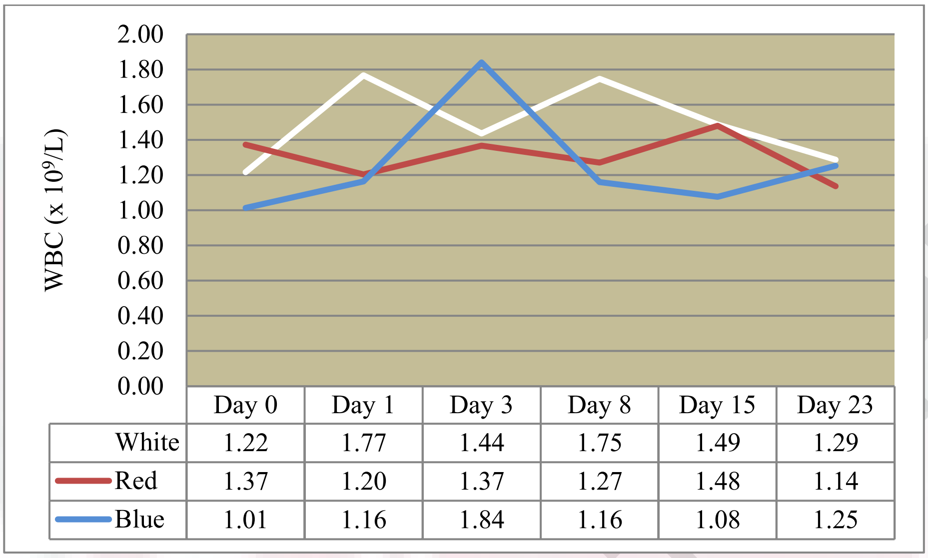


Figure 4.3: Effect of different light treatments of fish and their resulting hemoglobin throughout 23 days of study.



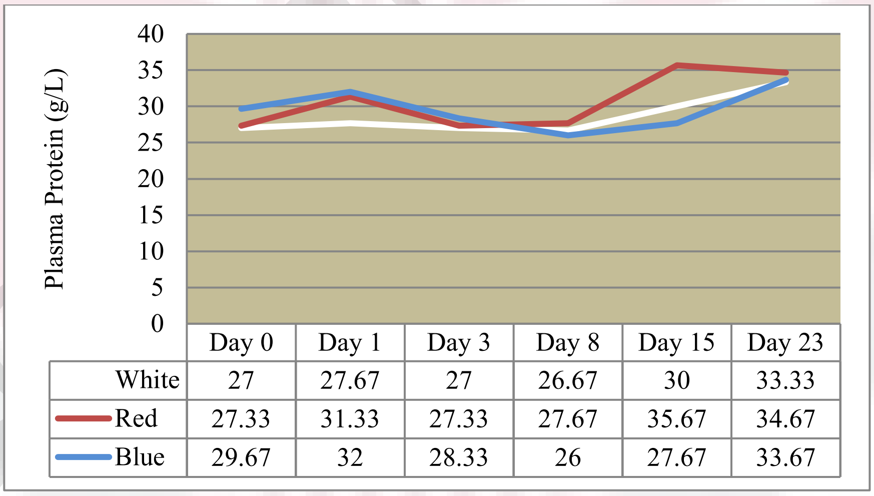
	Day 0	Day 1	Day 3	Day 8	Day 15	Day 23
White	114.33	114.33	101.93	101.07	105.90	105.00
Red	101.87	101.87	100.63	112.33	108.27	105.67
Blue	99.33	99.33	111.67	102.00	93.47	111.67

Figure 4.4: Effect of different light treatments of fish and their resulting white blood cell count throughout 23 days of study.



	Day 0	Day 1	Day 3	Day 8	Day 15	Day 23
White	1.22	1.77	1.44	1.75	1.49	1.29
Red	1.37	1.20	1.37	1.27	1.48	1.14
Blue	1.01	1.16	1.84	1.16	1.08	1.25

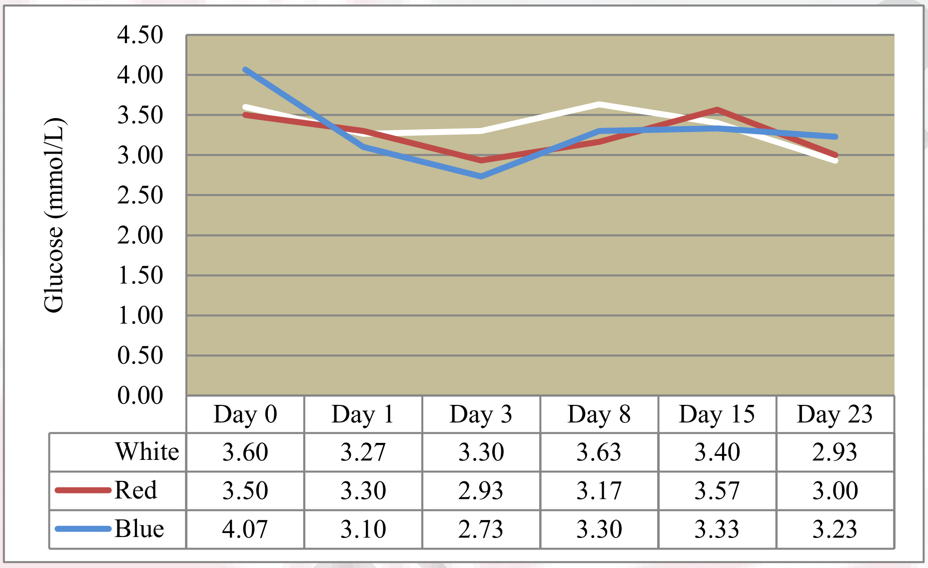
Figure 4.5: Effect of different light treatments of fish and their resulting plasma protein throughout 23 days of study.



	Day 0	Day 1	Day 3	Day 8	Day 15	Day 23
White	27	27.67	27	26.67	30	33.33
Red	27.33	31.33	27.33	27.67	35.67	34.67
Blue	29.67	32	28.33	26	27.67	33.67

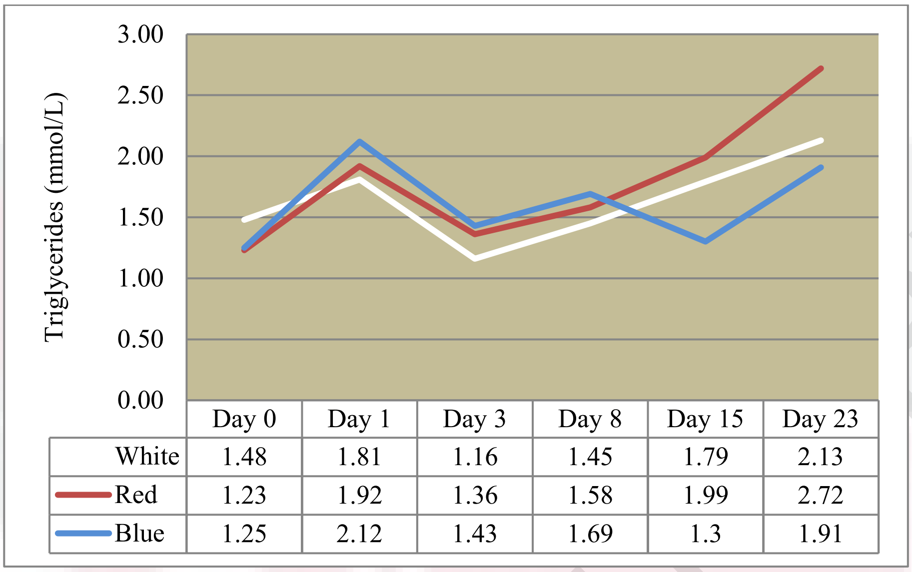
Glucose level among light treatments was not significantly different on day1, 3, 8, 15 and 23 even though all three colours showed decrease of glucose level on day 1 and 3 especially (blue colour dropped 23.83% from day 0 to day 1 and 12.05% from day 1 to day 3) (Figure 4.6).

Figure 4.6: Effect of different light treatments of fish and their resulting glucose level throughout 23 days of study.



Triglycerides levels of fish in all light treatments increased from day 0 to day 1 (22.3% for white light; 56.1% for red light; 69.6% for blue light), then decline from day 1 to day 3 (35.91% for white light; 29.17% for red light; 32.55% for blue light) before rising on last day of study (for 83.62% for white light; 100% for red light; 33.57% for blue light) (Figure 4.7).

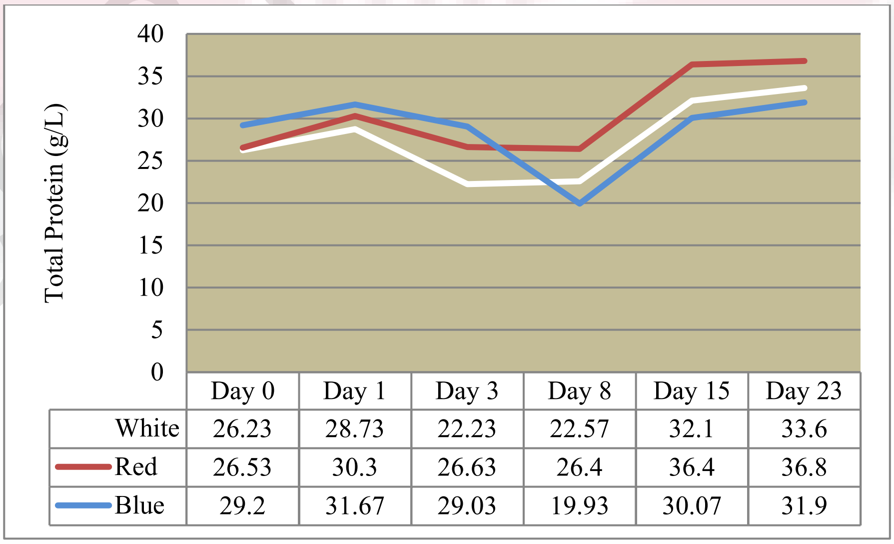
Figure 4.7: Effect of different light treatments of fish and their resulting triglycerides level throughout 23 days of study.



	Day 0	Day 1	Day 3	Day 8	Day 15	Day 23
White	1.48	1.81	1.16	1.45	1.79	2.13
Red	1.23	1.92	1.36	1.58	1.99	2.72
Blue	1.25	2.12	1.43	1.69	1.3	1.91

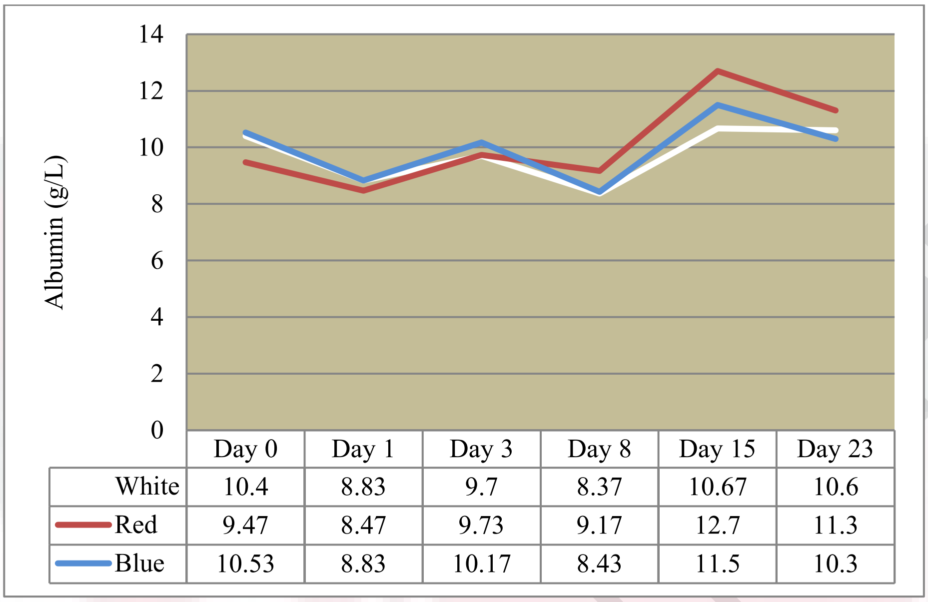
A similar trend was also observed on total protein level that showed a small rise on day 1, continue with small decrease on day 3 and increase again on day 23 for all light treatments (Figures 4.8, 4.9 and 4.10).

Figure 4.8: Effect of different light treatments of fish and their resulting total protein throughout 23 days of study.



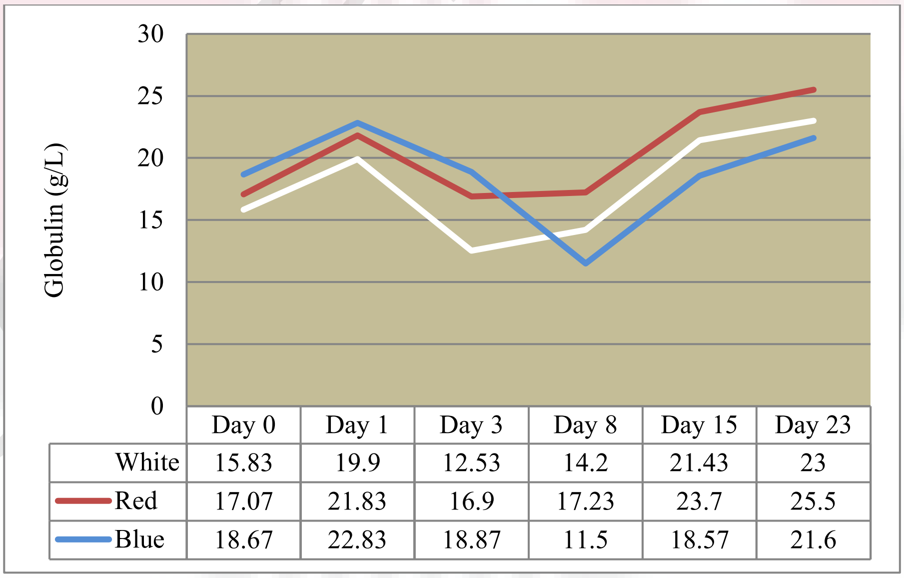
	Day 0	Day 1	Day 3	Day 8	Day 15	Day 23
White	26.23	28.73	22.23	22.57	32.1	33.6
Red	26.53	30.3	26.63	26.4	36.4	36.8
Blue	29.2	31.67	29.03	19.93	30.07	31.9

Figure 4.9: Effect of different light treatments of fish and their resulting albumin level throughout 23 days of study.



	Day 0	Day 1	Day 3	Day 8	Day 15	Day 23
White	10.4	8.83	9.7	8.37	10.67	10.6
Red	9.47	8.47	9.73	9.17	12.7	11.3
Blue	10.53	8.83	10.17	8.43	11.5	10.3

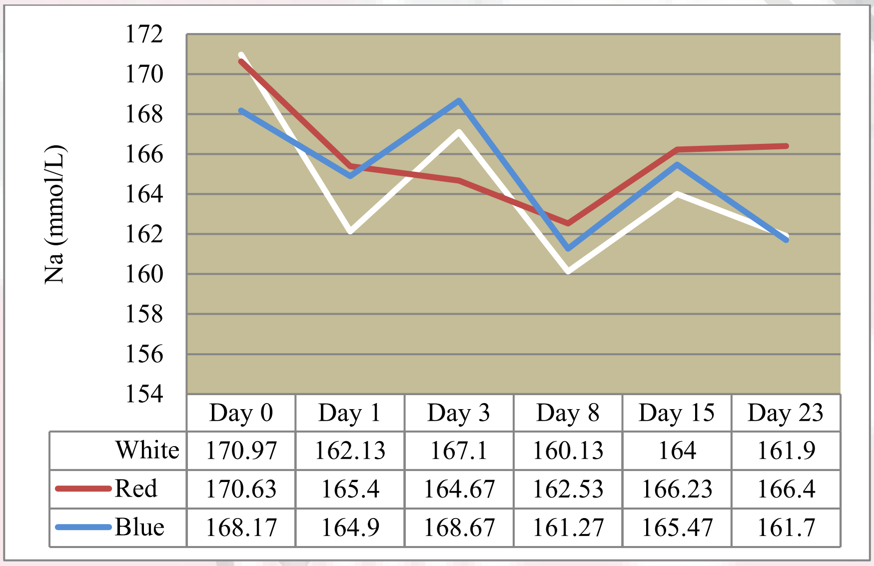
Figure 4.10: Effect of different light treatments of fish and their resulting globulin level throughout 23 days of study.



	Day 0	Day 1	Day 3	Day 8	Day 15	Day 23
White	15.83	19.9	12.53	14.2	21.43	23
Red	17.07	21.83	16.9	17.23	23.7	25.5
Blue	18.67	22.83	18.87	11.5	18.57	21.6

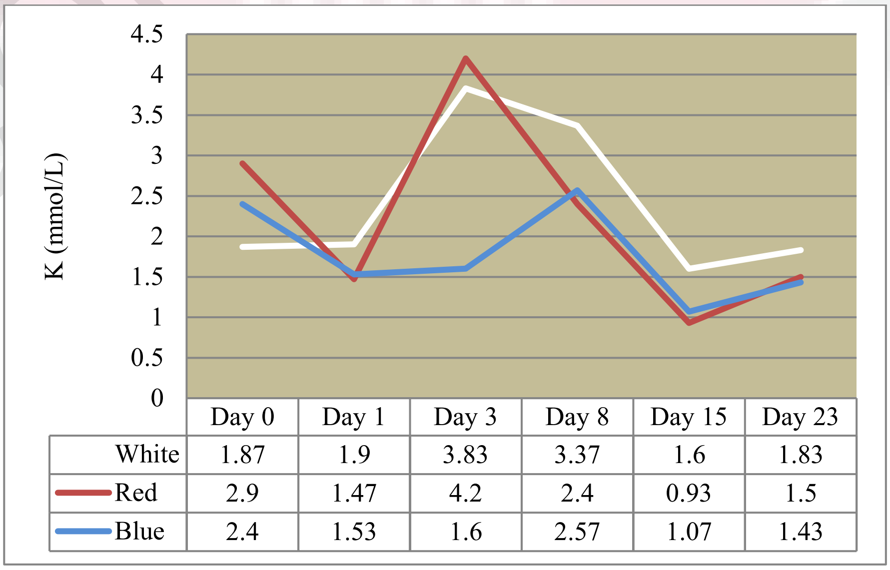
The major ions (Na, K, Cl) showed fluctuation from day 1 to day 23, with no significant different found among fish treated with the three colours of light treatment (Figures 4.11, 4.12 and 4.13).

Figure 4.11: Effect of different light treatments of fish and their resulting sodium (Na) throughout 23 days of study.



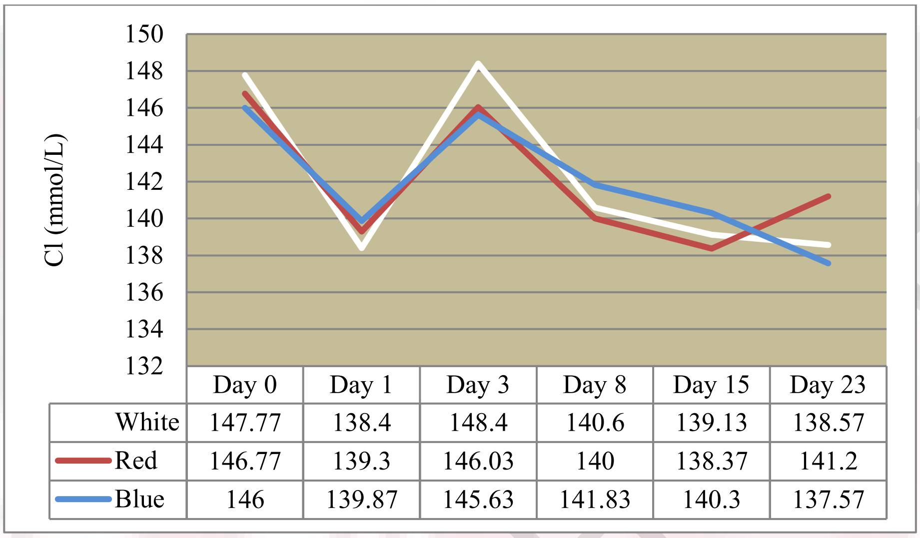
	Day 0	Day 1	Day 3	Day 8	Day 15	Day 23
White	170.97	162.13	167.1	160.13	164	161.9
Red	170.63	165.4	164.67	162.53	166.23	166.4
Blue	168.17	164.9	168.67	161.27	165.47	161.7

Figure 4.12: Effect of different light treatments of fish and their resulting potassium (K) throughout 23 days of study.



	Day 0	Day 1	Day 3	Day 8	Day 15	Day 23
White	1.87	1.9	3.83	3.37	1.6	1.83
Red	2.9	1.47	4.2	2.4	0.93	1.5
Blue	2.4	1.53	1.6	2.57	1.07	1.43

Figure 4.13: Effect of different light treatments of fish and their resulting chloride (Cl) throughout 23 days of study.



	Day 0	Day 1	Day 3	Day 8	Day 15	Day 23
White	147.77	138.4	148.4	140.6	139.13	138.57
Red	146.77	139.3	146.03	140	138.37	141.2
Blue	146	139.87	145.63	141.83	140.3	137.57

4.2 Growth performance

Blue light showed a significant difference on juvenile red tilapias' length gains as compared to white light (Figure 4.14). Although all the colours did not show significant difference on body weight gain, specific growth rate and feed conversion rate (Table 4.6), these parameters presented their best value for fish under blue light, followed by white and red lights (Figures 4.15, 4.16 and 4.17).

Table 4.6: Growth performance of juvenile red tilapia after 23 days of different light treatments.

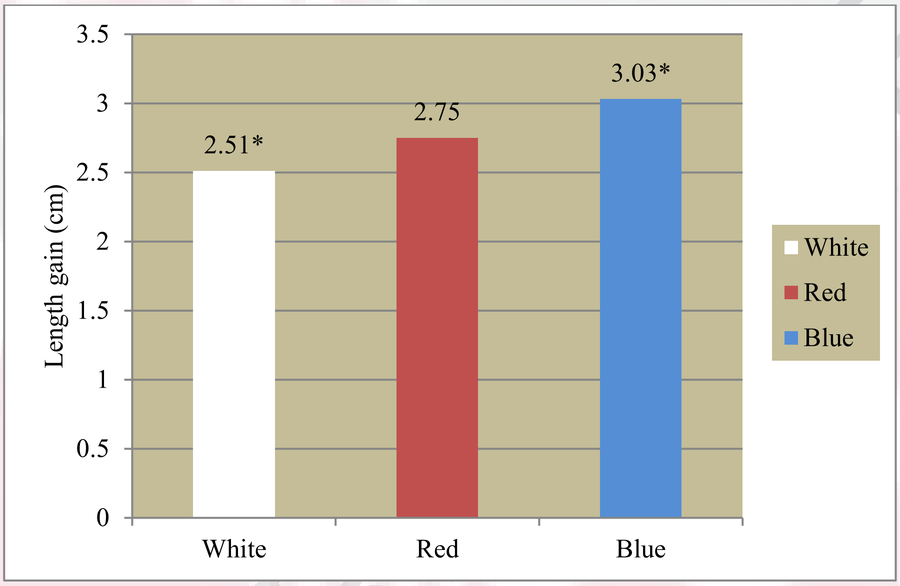
Light treatments (number of fish)	White (n=15)	Red (n=15)	Blue (n=15)	Sig.	
Initial length (cm)	8.84±0.27	8.47±0.22	8.61±0.25	0.574	NS
Final length (cm)	11.35±0.20	11.21±0.31	11.65±0.21	0.453	NS
Initial weight (g)	12.80±1.12	11.81±0.87	12.45±0.77	0.748	NS
Final weight (g)	24.56±1.29	22.76±1.65	24.46±1.18	0.593	NS
Average length gain (cm)	2.51±0.10*	2.75±0.10	3.03±0.10*	0.002	S
Body weight gain (g)	11.76±0.57	10.95±0.89	12.01±0.75	0.58	NS
Feed conversion rate	1.17±0.04	1.38±0.15	1.18±0.07	0.234	NS
Specific growth rate (%/day)	2.95±0.18	2.83±0.12	2.98±0.17	0.782	NS

The table shows mean±SEM of three replicate tanks per treatment.

S= significant difference; NS= no significant difference.

* denotes P value <0.05.

Figure 4.14: Average length gain of juvenile red tilapia after 23 days of different light treatments.



* denotes P value < 0.05.

Figure 4.15: Average body weight gain of juvenile red tilapia after 23 days of different light treatments.

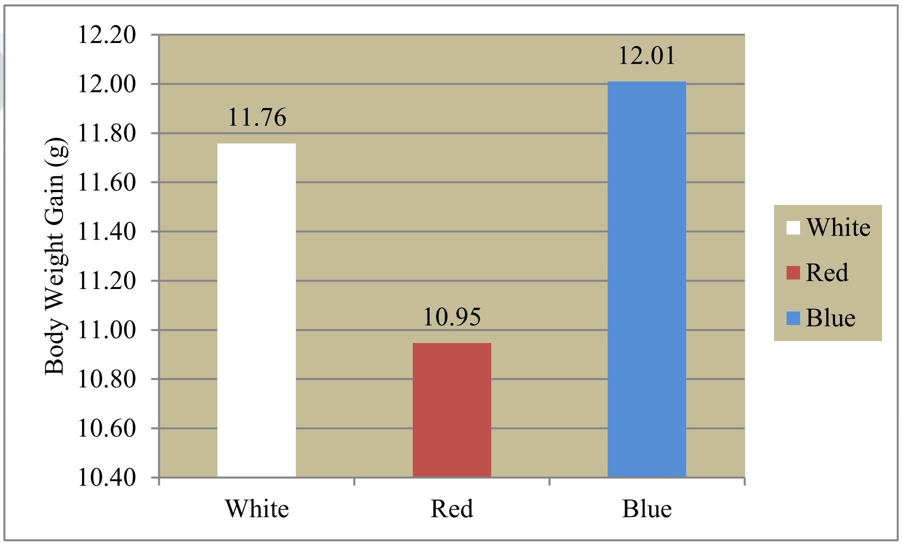


Figure 4.16: Feed conversion rate of juvenile red tilapia after 23 days of different light treatments.

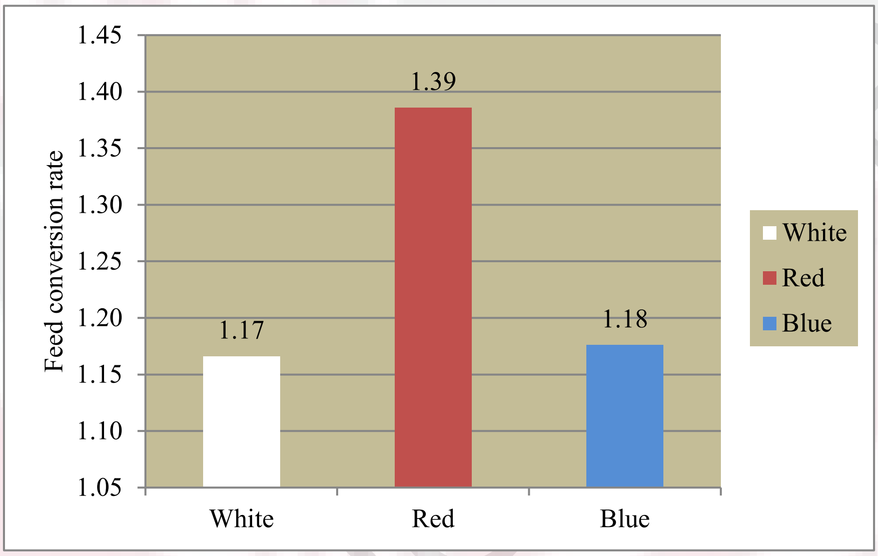
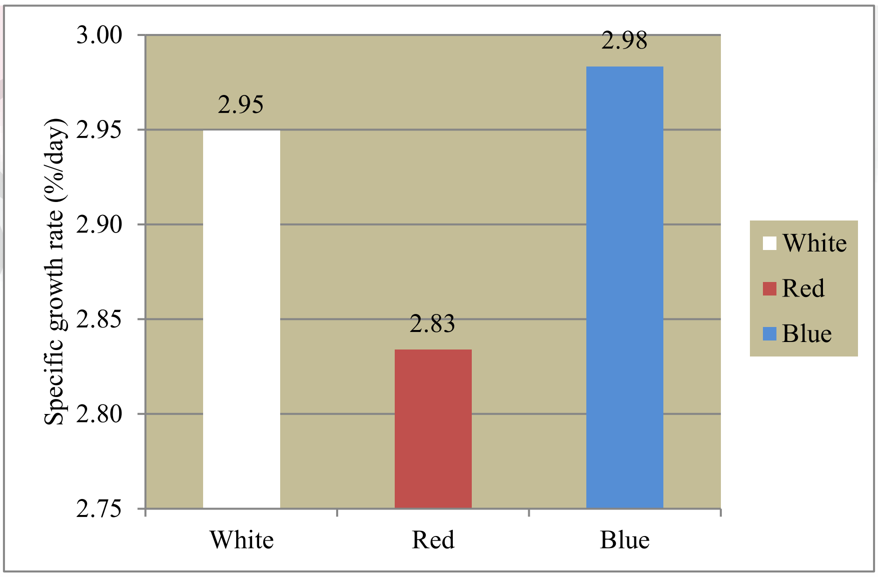


Figure 4.17: Specific growth rate of juvenile red tilapia after 23 days of different light treatments.



Discussion

In this study, the hematological parameters and plasma biochemistry of juvenile red tilapia were examined to detect the effect of blue and red LED lightings towards stress response in fish. In addition, the length and weight of juvenile red tilapia were evaluated after 23 days of study and calculated the average length gain, body weight gain, specific growth performance and feed conversion rate as growth performance parameters.

There was no significant difference in fish treated with blue and red lights in terms of all hematological parameters (RBC count, hemoglobin, MCH, MCHC, PCV, WBC, thrombocyte and plasma protein) although blue light appeared to have slightly high PCV on first 8 days of experiment. The differences on RBC count, hemoglobin, PCV, WBC count and plasma protein among light treatments on sampling days could be due to other extrinsic factors including water quality changes (Mirea, 2013 ; Latif and Iqbal, 2015) that was not well controlled in this experiment. The lack of insignificant difference on hematological parameters among light treatments was also reported by Eslamloo et al. (2015) who found no effect of 8 weeks light treatments on hematological indices of goldfish. Similarly, hematological indices of rainbow trout did not show significant differences after 111 days of light treatments although significant interaction of plasma cortisol was observed (Karakatsouli et al., 2008). This suggests that treatment with different colours of lights fail to induce hematological parameters changes even in long period of experiment. Hematological parameters could provide reliable information on metabolic disorder, deficiencies and chronic stress status (Iwama et al., 1999). The absence of significant difference of hematological parameters between colours of light in this study suggest that there are no significant difference on metabolic disorder, deficiencies and chronic stress status among red, blue and white lights.

The difference of plasma biochemistry parameters (triglycerides, glucose, total protein, albumin, globulin, major ions- Na, K, Cl) among red, blue and white LED lights were also not significant. These results suggest that blue and red lights did not have effect on plasma biochemistry which including plasma glucose. This finding is similar to the study done by Eslamloo et al. (2015) and Imannpoor et al. (2011) where plasma glucose did not differ among light treatments in experiment with goldfish and juvenile Caspian Kutum. Although plasma glucose is known to be one of the stress indicators, a study suggested that no significant changes in plasma glucose may be observed even though the fish is under stress (Marcel et al., 2009). This is probably due to rapid consumption of glucose during stress in order to maintain homeostasis. In this study, glucose level is measured after one day of experiment instead of few hours after treatment. Hence, there might be presence of rapid glucose consumption to cope with stress in the 24 hours, leading to the low glucose level on day 1 and day 3 of experiment.

With the absence of significant difference in both haematological parameters and plasma biochemistry, the study suggested that there was no secondary stress response induced by red and blue lights.

In contrast to previous studies that found red light favor the growth performance in different species of fish (Karakatsouli et al., 2008; Head and Malison, 2000; Volapato et al., 2013), the current study suggested that blue light have impact on length gains of juvenile red tilapia. This could be explained by different spectral sensitivity of retina among fish species living in different ecology (Partridge and Cummings, 1999; Hornsby et al., 2013). More studies could be done in future to understand more about spectral sensitivity of juvenile red tilapia and the benefit of using blue light on the fish.

Since there was also no significant difference seen between body weight gain, specific growth rate and feed conversion rate of juvenile red tilapia among the light treatments, the significant length gained by blue light as compared to white light suggests that effect of light treatments on growth potential could not be seen clearly in the short period of less than 1 month experiment. In another study, colours of light did stimulate feeding motivation but did not show significant improvement of growth (Volpato et al., 2013). In another similar study with light treatments done on yellow perch for 21 days experiment, the overall growth potential was insignificant although different colours of light did favour length gains (Head and Malison, 2000). In another experiment of 111 days, different colours of light led to significantly higher total length compared with normal white light but insignificant different for other growth parameters (body weight gain, specific growth rate and feed conversion rate) in rainbow trout (Karakatsouli et al., 2008). These suggest that longer period of experiment of more than 4 months should be carried out in order to prove the better growth potential by different colours of light.

As artificial light become more important in intensive fish farming, a better understanding on the effect of light spectral is required. Current study found no metabolic disorder, deficiencies and chronic stress induced by red and blue lights in juvenile red tilapia. This gives chance for wide spectral of light to be used in the rearing of red tilapia. With the significant length gains by blue light as compared to white light, more study should be done in future to evaluate the better benefit of blue light in this species.

Conclusion

The use of red and blue lights do not have any effect on hematological parameters and plasma biochemistry result as compared to the white light throughout 23 days of experiment. Hence, both colours do not imply change on juvenile red tilapia's stress response when compared to normal white light.

The blue light appeared to provide better length gains as compared to white light after 23 days of experiment. Although both red and blue lights do not have significant difference on other growth parameters (body weight gain, specific growth rate and feed conversion rate) as compared to white light, but blue light showed better result among three groups.

In conclusion, there is no stress response induced by different colours of light and blue light could be suggested as the better colour of choice for juvenile red tilapia to improve body length.

Recommendation

This project was a preliminary study to understand effect of colours of light on stress response and growth performance of juvenile red tilapia. More studies should be done on different choices of colours (orange, yellow, green and violet) of light and other parameters of fish physiology (behavior and reproduction).

The finding of higher length gained by blue light treatment, more studies on spectral sensitivity of red tilapia should be done to determine the possibility of blue light be the most sensitive colour in vision of juvenile red tilapia.

The study of stress response should also include plasma cortisol evaluation. Cortisol and glucose could not be eliminated from the stress indicators list especially for evaluation of acute stress response. However for chronic experiment, cortisol may be reported as complementary data together with measurements such as other stress hormones, hsps and blood-cell counts (Marcel et al., 2009).

Further studies could be done with longer period of experiment in order to detect significant difference on growth performance of red tilapia. The period suggested is more than 4 months.

In addition, a better experimental design could bring a more promising and accurate result for the study. There should be an increase of sample size in order to decrease random sampling error. Additionally, better control on other extrinsic factors such as water quality (include salinity, temperature, dissolved oxygen and nitrogen) should be given more emphasis.

References

Ainsworth, A. J., Dexiang, C., & Waterstrat, P. R. (1991). Changes in peripheral blood leukocyte percentages and function of neutrophils in stressed channel catfish. *Journal of Aquatic Animal Health*, *3* (1), 41-47.

Barcellos, L. J. G., Ritter, F., Kreutz, L. C., Silva, L. B. D., Cericato, L., & Quevedo, R. M. (2006). The color of illumination affects the stress response of jundiá (Rhamdia quelen, Quoy & Gaimard, Heptapteridae). *Ciência Rural*, *36* (4), 1249-1252.

Barton, B. A. (2002). Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and comparative biology*, *42* (3), 517-525.

Boeuf, G., & Le Bail, P. Y. (1999). Does light have an influence on fish growth?. *Aquaculture*, *177* (1), 129-152.

Chen, W. H., Sun, L. T., Tsai, C. L., Song, Y. L., & Chang, C. F. (2002). Cold-stress induced the modulation of catecholamines, cortisol, immunoglobulin M, and leukocyte phagocytosis in tilapia. *General and comparative endocrinology*, *126* (1), 90-100.

Clauss, T. M., Dove, A. D., & Arnold, J. E. (2008). Hematologic disorders of fish. *Veterinary clinics of North America: Exotic animal practice*, *11* (3), 445-462.

Douglas, R. H., & Hawryshyn, C. W. (1990). Behavioural studies of fish vision: an analysis of visual capabilities. In *The visual system of fish* (pp. 373-418). Springer Netherlands.

Ekstrzm, P., & Meissl, H. (1997). The pineal organ of teleost fishes. *Reviews in Fish Biology and Fisheries*, *7* (2), 199-284.

Eslamloo, K., Akhavan, S. R., Eslamifar, A. & Henry, M. A. (2015), Effects of background colour on growth performance, skin pigmentation, physiological condition and innate immune responses of goldfish, *Carassius auratus*. *Aquaculture Research, 46*, 202–215.

Imanpoor, M. R., Enayat Gholampour, T., & Zolfaghari, M. (2011). Effect of light and music on growth performance and survival rate of goldfish (Carassius auratus). *Iranian Journal of Fisheries Sciences*, *10* (4), 641-653.

Iwama, G. K., Vijayan, M. M., Forsyth, R. B., & Ackerman, P. A. (1999). Heat shock proteins and physiological stress in fish. *American Zoologist*,*39* (6), 901-909.

Jerlov, N.G., (1968). *Optical Oceanography*. Elsevier, Amsterdam.

Johnson, E. O., Kamilaris, T. C., Chrousos, G. P., & Gold, P. W. (1992). Mechanisms of stress: a dynamic overview of hormonal and behavioral homeostasis. *Neuroscience & Biobehavioral Reviews*, *16* (2), 115-130.

Giustina, A., & Wehrenberg, W. B. (1992). The role of glucocorticoids in the regulation of growth hormone secretion mechanisms and clinical significance. *Trends in Endocrinology & Metabolism*, *3* (8), 306-311.

Head, A. B., & Malison, J. A. (2000). Effects of lighting spectrum and disturbance level on the growth and stress responses of yellow perch *Perca flavescens*. *Journal of the World Aquaculture Society*, *31* (1), 73-80.

Hornsby, M. A., Sabbah, S., Robertson, R. M., & Hawryshyn, C. W. (2013). Modulation of environmental light alters reception and production of visual signals in Nile tilapia. *The Journal of experimental biology*, *216* (16), 3110-3122.

Karakatsouli, N., Papoutsoglou, S. E., Panopoulos, G., Papoutsoglou, E. S., Chadio, S., & Kalogiannis, D. (2008). Effects of light spectrum on growth and stress response of rainbow trout *Oncorhynchus mykiss* reared under recirculating system conditions. *Aquacultural Engineering, 38* (1), 36-42.

Karakatsouli, N., Katsakoulis, P., Leondaritis, G., Kalogiannis, D., Papoutsoglou, S. E., Chadio, S., & Sakellaridis, N. (2012). Acute stress response of European sea bass *Dicentrarchus labrax* under blue and white light. *Aquaculture*, *364*, 48-52.

Latif, M., Ali, M., & Iqbal, F. (2015). Seasonal variations in hematological and serum biochemical profile of *Channa marulius* are complementary to the changes in water quality parameters of River Chenab in Pakistan. *Pakistan Journal of Zoology*, *47* (6), 1699-1707.

Law, W. Y., Chen, W. H., Song, Y. L., Dufour, S., & Chang, C. F. (2001). Differential in vitro suppressive effects of steroids on leukocyte phagocytosis in two teleosts, tilapia and common carp. *General and Comparative Endocrinology*, *121* (2), 163-172.

Maia, C. M., & Volpato, G. L. (2013). Environmental light color affects the stress response of Nile tilapia. *Zoology*, *116* (1), 64-66.

Marcel Martinez-P., Luis, R.M. and Rogelio, R. (2009). Cortisol and glucose: Reliable biomarkers of fish stress. *Pan. Am. J. Aquatic Sce., 4 (2)*, 158-178.

Marchesan, M., Spoto, M., Verginella, L., & Ferrero, E. A. (2005). Behavioural effects of artificial light on fish species of commercial interest. *Fisheries Research, 73* (1–2), 171-185.

Maule, A. G., & Schreck, C. B. (1990). Changes in numbers of leukocytes in immune organs of juvenile coho salmon after acute stress or cortisol treatment. *Journal of Aquatic Animal Health*, *2* (4), 298-304.

Mazuki, H., (2015). Industry and Market Status of Tilapia in Malaysia. 4th International Trade And Technical Conference And Exposition On Tilapia (Tilapia 2015).

McDonald, G., & Milligan, L. (1997). Ionic, osmotic and acid-base regulation in stress. *Fish stress and health in aquaculture*, *62*, 119-145.

Migaud, H., Cowan, M., Taylor, J., & Ferguson, H. W. (2007). The effect of spectral composition and light intensity on melatonin, stress and retinal damage in post-smolt Atlantic salmon, *Salmo salar*. *Aquaculture*, *270* (1), 390-404.

Mirea, C. C. (2013). Hematological characterization of Nile tilapia (*Oreochromis niloticus*, Linnaeus, 1758) reared intensively in a recirculating aquaculture system in relation to water temperature. *Scientific Papers Animal Science and Biotechnologies*, *46* (2), 234-237.

Mommsen, T. P., Vijayan, M. M., & Moon, T. W. (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries*, *9* (3), 211-268.

Owen, M. A., Davies, S. J., & Sloman, K. A. (2010). Light colour influences the behaviour and stress physiology of captive tench (Tinca tinca). *Reviews in Fish Biology and Fisheries*, *20* (3), 375-380.

Partridge, J. C. (1990). The colour sensitivity and vision of fishes. *Light and Life in the Sea*, 167-184.

Partridge, J. C., & Cummings, M. E. (1999). Adaptation of visual pigments to the aquatic environment. In *Adaptive mechanisms in the ecology of vision* (pp. 251-283). Springer Netherlands.

Patnaik, L., Raut, D., Swain, A., Mohanty, B., Swain, S. & Nayak, A., (2014). Study on variation in fish length, weight and protein ratio based on feed in Tilapia sp. and Anabas sp. *European Journal of Zoological Research, 3 (2),* 23-27

Peres, H., Costas, B., Perez-Jimenez, A., Guerreiro, I., & Oliva-Teles, A. (2015). Reference values for selected hematological and serum biochemical parameters of Senegalese sole (*Solea senegalensis* Kaup, 1858) juveniles under intensive aquaculture conditions. *Journal of Applied Ichthyology*, *31* (1), 65-71.

Perez-Sanchez, J., & Le Bail, P. Y. (1999). Growth hormone axis as marker of nutritional status and growth performance in fish. *Aquaculture*, *177* (1), 117-128.

Placinta, S., Victor, C., Tiberiu, C. M., & Mihai, P. S. (2014). Hematological profile of juvenile European catfish (*Silurus glanis*) reared under different stocking densities in recirculating system conditions. *Scientific Papers Animal Science and Biotechnologies*, *47* (1), 246-251.

Pottinger, T. G. (2008). The stress response in fish-mechanisms, effects and measurement. *Fish Welfare. Blackwell Publishing Ltd, UK*, 32-48.

Randall, D. J., & Perry, S. F. (1992). Catecholamines. *In* W. S. Hoar and D. J. Randall (eds.), *Fish physiology,* Vol. 12B, pp. 255–300. Academic Press, New York.

Reid, S. G., Bernier, N. J., & Perry, S. F. (1998). The adrenergic stress response in fish: control of catecholamine storage and release. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, *120* (1), 1-27.

Schreck, C. B., Olla, B. L., & Davis, M. W. (1997). Behavioral responses to stress. *Fish stress and health in aquaculture*, *62*, 145-170.

Sierra-Flores, R., Davie, A., Grant, B., Carboni, S., Atack, T., & Migaud, H. (2016). Effects of light spectrum and tank background colour on atlantic cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) larvae performances. *Aquaculture, 450*, 6-13.

Tamazouzt, L., Chatain, B., & Fontaine, P. (2000). Tank wall colour and light level affect growth and survival of Eurasian perch larvae (*Perca fluviatilis* L.). *Aquaculture, 182* (1–2), 85-90.

Tort, L., & Teles, M. (2011). *The Endocrine Response to Stress-A Comparative View*. INTECH Open Access Publisher.

Van Weerd, J. H., & Komen, J. (1998). The effects of chronic stress on growth in fish: A critical appraisal. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *120* (1), 107-112.

Very, N. M., & Sheridan, M. A. (2007). Somatostatin regulates hepatic growth hormone sensitivity by internalizing growth hormone receptors and by decreasing transcription of growth hormone receptor mRNAs. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *292* (5), R1956-R1962.

Vijayan, M. M., & Moon, T. W. (1992). Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*).*Canadian Journal of Fisheries and Aquatic Sciences*, *49* (11), 2260-2266.

Villamizar, N., Vera, L. M., Foulkes, N. S., & Sánchez-Vázquez, F. J. (2014). Effect of lighting conditions on zebrafish growth and development. *Zebrafish*, *11* (2), 173-181.

Volpato, G. L., & Barreto, R. E. (2001). Environmental blue light prevents stress in the fish Nile tilapia. *Brazilian Journal of Medical and Biological Research*, *34* (8), 1041-1045.

Volpato, G. L., Bovi, T. S., de Freitas, R. H., da Silva, D. F., Delicio, H. C., Giaquinto, P. C., & Barreto, R. E. (2013). Red Light Stimulates Feeding Motivation in Fish but Does Not Improve Growth. *PloS one*, *8* (3).

Appendix

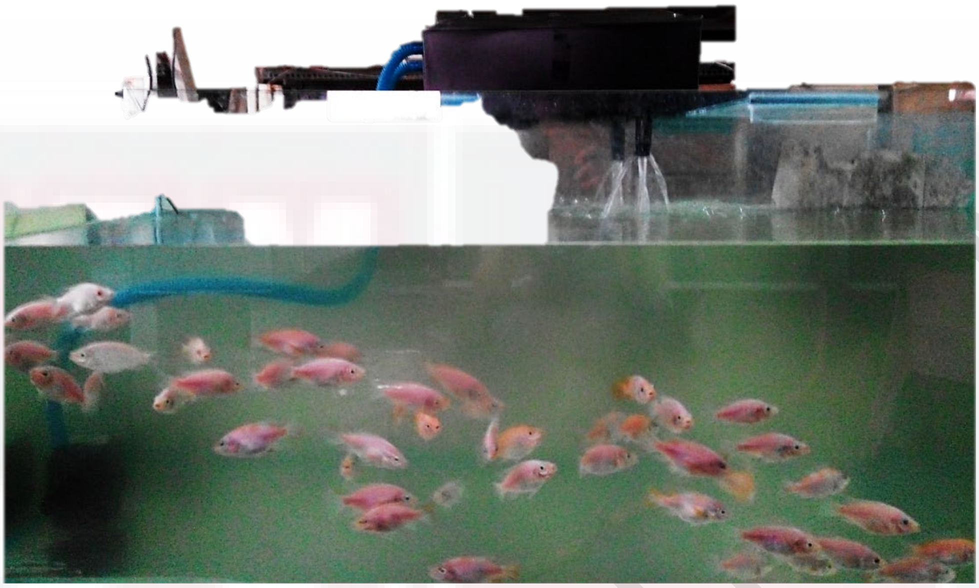


Figure 8.1 Juvenile red tilapia in one of the tanks during acclimatization period.

Figure 8.2 Tanks with red, blue (treatment) and white (control) lights on.

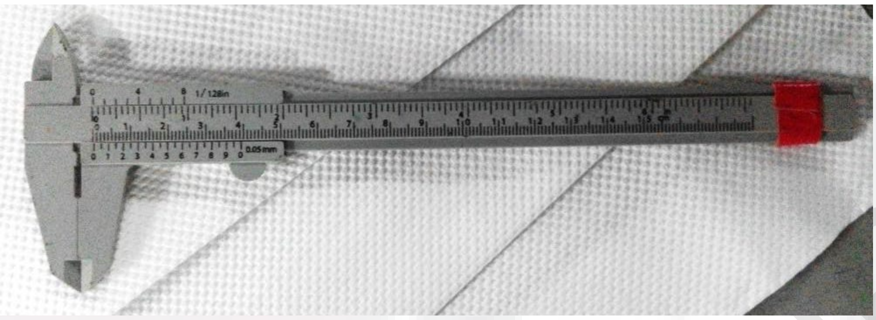


Figure 8.3 Length of juvenile red tilapia was measured using Vernier caliper.

Figure 8.4 Weigh was measured using weighing scale.

Figure 8.5 Blood was withdrawn using 1mL syringe and 25G hypodermic needle from caudal vein puncture.