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Enhancement of body performance and growth performance of juvenile mahseer (*Tor soro*) using differently colored containers

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Abstract

Mahseer (*Tor soro*) growth performance tends to be slow, necessitating further development and intensification of cultivation. One way to develop aquaculture intensification is to manipulate cultivation containers to create optimal environmental conditions for the mahseer to grow. This study aimed to examine the body performance and growth performance of mahseer reared in different colored containers. Experimental research with completely randomized design was employed, with four colored container treatments namely treatment A (transparent), B (green), C (blue), and D (black), with four replications in each treatment. Findings indicate that different rearing media colors had significant effect on absolute length (4.68 ± 0.24 cm), absolute weight (1.58 ± 0.35 g), specific growth rate (2.17 ± 0.38%), feed conversion ratio (2.87 ± 0.04), survival rate (100 ± 0.00%), gross energy (3,816 ± 65.05 cal/g), and body proximate. Physiologically, mahseer fish bred using blue and black containers tend to be more resistant to stress.The best body performance and growth performance were observed in the blue and black colored containers.

Keywords: Body performance, Growth, Mahseer, *Tor soro*

Introduction

Mahseer (*Tor soro*) and several other types of tor fish are gradually decreasing in number (Haser et al., 2022). Such a decrease in *Tor soro* is the result of overfishing and substantial damages to its original habitat (Muchlisin et al., 2022). The cultivation of *Tor soro* faces several obstacles such as a slow growth rate and difficulties in adapting to the environment (compared to exotic fish) (Mohapatra et al., 2017). Another obstacle is the high mortality rate due to environmental stress, especially in

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the juvenile stage (Khan et al., 2017). In addition to controlled environmental elements such as water quality, increased growth performance can also be achieved through coloring the rearing media (containers). Such color manipulation in culture is essential, especially in reducing stress (McLean, 2021).

The container's color manipulation influences the water's light intensity so that the amount of light penetrating rearing media could affect visual feeding (Rahmawati & Kadarini, 2018). McLean (2021), mentioned that colors can represent a fish's original environment affecting fish physiology, body color, and behavior. He further adds that the use of container colors must be adjusted to conform to the characteristics of cultivated fish. These accounts suggest that each type of fish will represent the color of the container according to its properties and characteristics, including mahseer. In fish culture, several studies have been conducted in regards to container color manipulations, such as black-colored container to increase the growth of peled fish Coregonus (Sebesta et al., 2018), blue for increasing growth and larval synthesis of tilapia (Brian, 2015), black for increasing growth of larvae and seeds of *Macrobranchium amazonicum* (Mallasen & Valenti, 2007), cream in rainbow fish *Onchorhynchus mykiss* (Üstündag & Rad, 2015), and yellowfin tuna (*Thunnus thynnus*) (Yúfera et al., 2014). Furthermore, according to McLean (2021), fish kept in dark colored containers have better growth than fish kept in light colored containers because fish kept in light colored containers tend to have higher cortisol levels than fish kept in dark colored containers, meaning that fish tend to be more susceptible to stress in brightly colored containers, and this will cause growth to be disrupted because stressful conditions can inhibit the fish's endocrine system and result in inhibited growth hormone secretion.

These findings indicate that rearing media's color is one of the environmental factors that play a role in stimulating physiological stress especially in diurnal fish with schooling behaviour such as mahseer. The appropriate cultivation model provides the opportunity to reduce stress and in turn indirectly affects fish behavior (Kalkhundiya et al., 2021). This study was conducted to obtain the best color (of rearing media) to support the synthesis and growth performance of mahseer (*Tor soro*), with the major purpose of selecting the best container color(s) as the promising production technology for mahseer's intensive cultivation.

Materials and Methods

Time and place of study

This research was conducted at the Fish Hatchery and Farming Laboratory, Nutfah Plasma Installation, Center for Freshwater Aquaculture Fisheries (BBPBAT) from March–July 2021. Water quality measurements were carried out at the BBPBAT Environmental Toxicology Laboratory and the Environmental Aquaculture Laboratory of the Department of Aquaculture, Faculty of Fisheries and Marine Sciences (FPIK), IPB University. While the gross energy and proximate analysis was performed at the IPB Inter University Central Laboratory.

Experimental conditions

The study used completely randomized design with four treatments (container colors), and four replicates. The color treatments were colorless (transparent), green with the colorimeter code L* 43.77 Toca Color 4215, blue with the colorimeter code L* 25.46.09 Toca Color 3304, and black with the colorimeter code L* 25.39 Toca Color 5318.

Experimental fish and rearing media (containers)

The fish samples were Mahseer (*Tor soro*) seeds with an average length of 3.48 ± 0.07 cm and an average weight of 0.60 ± 0.02 g. The juveniles were hatched from artificial spawning at the Freshwater Fishery Nutfah Plasma Research Installation, Cijeruk, Bogor. The rearing media were 16 aquariums measuring 48 cm × 48 cm × 30 cm, equipped with aeration stones with 2 cm in diameter and 3 cm in length. The aquariums were washed, disinfected by chlorine, and dried for three days. The aquariums were then wrapped with paperboardwhose color was measured using a colorimeter CR-400 (Konica Minolta, Osaka, Japan) and a Toca Color Finder (TCF). These aquariums had no recirculation system, with water volume of 40 L each. The stocking density was 1 individual/L (40 individuals per aquarium). Juvenile mahseer were fed PF1000 pellets commercial feed three times a day at 7:00 a.m., 12:00 p.m., and 5:00 p.m. The experimental period lasted for 80 days.

Blood profile and blood glucose

Blood was collected by cutting the tail with 0.1 mL as the sample; and it was taken from the venous caudalis using a syringe. Blood was sampled to measure blood profile and blood glucose, and measurements were carried out at 80 days of mahseer fish rearing. Three individuals per replicate were taken randomly and previously anesthetized using tricaine MS-222 at a dose of 75 mg/L. Blood was taken using a syringe (which had been rinsed with 1 mL of 3.8% sodium citrate) through caudalis vein and immediately centrifuged at 1,409×g for 10 minutes. The

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supernatant was also collected for biochemical plasma measurement. The overall plasma was collected on a polyethylene tube, which was then labeled and stored at –23℃ before being analyzed. Plasma glucose was determined using Glucose Oxsidase–Peroxidase Aminoantypirin (GOD-PAP) to examine colorimetric enzymatic of the commercial kit glucose liquicolor (Human mbH, Wiesbaden, Germany), as per the procedure described in the kit. Absorbance measurement was performed by a spectrophotometer with a wavelength of 500 nm.

Gross energy and proximate composition analysis

Gross energy values were obtained from the results of testing fish samples using the Parr 6200 bomb calorimeter method. Moisture content was measured using 1 g of sample weighed in a bowl, heated in the oven at 105℃ for 8 hours, and followed by weighing the moisture content. Ash content was measured by placing 1 g of sample in a porcelain crucible, burned until it was not smokey, then ignited in a furnace at 600℃ for 6 hours. Protein content was measured by adding 0.25 gram sample to a 100 mL Kjeldahl flask, along with 0.25 grams of selenium and 3 mLof concentrated H_2SO_4. It was heated to boil (destructed) for 1 hour until the solution is clear/colorless. Upon cooling, 50 mL of Aqua Dest and 20 mL of 40% NaOH was added to it before being distilled. The distillation results were poured into an Erlenmeyer flask containing a mixture of 2% of 10 mL of H_3BO_3 and 2 drops of pink Brom Cresol Green-Methyl Red indicator. Once the distillate reached 10 mL and turned bluish green, the distillation was halted. It was then titrated with 0.1 N HCl until it turned pink. The measurement of crude fiber content was carried out by dissolving 1 gram of sample in 100 mL of H_2SO_4 to a concentration of 1.255, which was heated to a boil and dissolved for 30 minutes.. It was then filtered through a paper with the aid of a Buchner funnel. The filtered residue was rinsed with 20–30 mL of boiling water then successively rinsed with 25 mL of water along with 25 mL of 1.25% boiling H_2SO_4; three times for 2 hours. After cooling, the residue and porcelain crucible were weighed, then ignited in a furnace at 600℃ for 30 minutes, which later was cooled off and weighed again. The total carbohydrate was measured by the carbohydrate by difference method.

Sampling and data collection

Growth parameters were observed once in 20 days by measuring the fish body weight and total length. Fish fingerlings were sedated with an anesthetic solution to prevent fish stress during observation. Body weight was measured using a digital scale (accuracy: 0.1 mg), and total length was measured using a block micrometer (accuracy: 0.05 to 0.1 mm). The fish body samples were taken for proximate analysis, gross energy analysis, and blood profiles (erythrocytes, leukocytes, hemoglobin, hematocrit, and glucose) of Mahseer. Meanwhile, the specific growth rate (SGR), survival rate (SR), and feed conversion ratio (FCR) were calculated with the formula:

$$\mathrm{SGR}\ (\%/\mathrm{day}) = \frac{\mathrm{Ln}\ (\text{Final body weight}) - \mathrm{Ln}\ (\text{Initial body weight})}{\text{Duration of rearing period (days)}} \times 100$$

$$\mathrm{SR}\ (\%) = \frac{\text{Number of final survived larvae}}{\text{Number of initial stocked larvae}} \times 100$$

$$\mathrm{FCR} = \frac{\text{Total quantity of food intake}}{\text{Total weight during the whole rearing period}}$$

Water quality was measured every 2 days; dissolved oxygen (DO) was measured using Milwaukee; pH values were measured using Horiba pH 110; and turbidity was measured using Lutron TU-2016. while nitrites, nitrates, ammonia, total ammonia (total ammonia nitrogen [TAN], measured using the spectrophotometer method), alkalinity, and hardness (measured using a titrometer) were measured every 20 days. Water was changed at a maximum of 60% of the total volume of water every 5 days to maintain quality.

Statistical analysis

Growth parameters, namely SGR, SR, FCR, absolute weight, absolute length, final weight, and final length were analyzed with an analysis of variance (ANOVA) at 95% confidence level. If there were any significant difference, data were analyzed continuously using the Duncan's multiple range test (DMRT). For body proximate analysis, gross energy, blood profiles of Mahseer, the data were analyzed descriptively. The statistical analysis of the data was performed with Microsoft Excel and Minitab 19 softwares.

Results

Growth performance and body performance of juvenile mahseer

The growth of mahseer during hatchery measured by their weight and length is presented in Table 1. Before treatment,

Table 1. Growth and survival rate of juvenile mahseer reared in variously colored aquariums

Parameters	Aquarium color			
	A (Transparent)	B (Green)	C (Blue)	D (Black)
Initial length (cm)	3.51 ± 0.05[a]	3.44 ± 0.09[a]	3.41 ± 0.18[a]	3.56 ± 0.10[a]
Initial body weight (gr)	0.59 ± 0.01[a]	0.60 ± 0.06[a]	0.59 ± 0.08[a]	0.63 ± 0.05[a]
Final length (cm)	4.78 ± 0.15[a]	5.11 ± 0.21[bc]	5.54 ± 0.31[a]	5.37 ± 0.25[ab]
Final body weight (gr)	1.48 ± 0.14[a]	1.69 ± 0.19[a]	2.37 ± 0.37[b]	2.15 ± 0.21[b]
Absolute length (cm)	3.96 ± 0.09[a]	4.07 ± 0.10[ab]	4.68 ± 0.24[d]	4.25 ± 0.06[bc]
Absolute weight (gr)	0.59 ± 0.01[a]	0.76 ± 0.12[ab]	1.58 ± 0.35[d]	1.25 ± 0.06[c]
Specific growth rate (%)	1.17 ± 0.03[a]	1.37 ± 0.22[a]	2.17 ± 0.38[b]	1.83 ± 0.14[b]
Feed conversion ratio (FCR)	3.14 ± 0.08[b]	3.63 ± 0.10[c]	2.87 ± 0.04[a]	3.03 ± 0.09[b]
Survival rate	82.5 ± 8.42[a]	100 ± 0.00[b]	100 ± 0.00[b]	99.4 ± 1.25[b]

Numbers followed by the same letter indicate that no significant difference found in the Duncan test (DMRT) at α = 5%.

the initial length and weight (in each treatment) were relatively similar, with the average initial length of 3.48 ± 0.07 cm and the average initial weight of 0.60 ± 0.02 g. After treatment, mahseer exhibited increased growth with 4.78–5.54 cm as the final length, and 1.48–2.37 g as the final weight. Absolute length growth parameter ranged between 3.96–4.68 cm, absolute weight ranged from 0.59–1.58 g, and SGR ranged between 1.17%–2.17%. Table 1 further shows that the highest growth parameter in terms of absolute length and absolute weight was found in treatment C (blue), with 4.68 cm and 1.58 g respectively. Meanwhile, the highest SGR was found in treatment C (blue) at 2.17% and treatment D (black) at 1.83%. ANOVA and post hoc DMRT with 95% confidence interval revealed that rearing mahseer in aquariums of different colors showed significant difference ($p < 0.05$) in final length, final weight, absolute length, absolute weight, and SGR parameters.

Body performance of mahseer can be examined by FCR and SR. As Table 1 demonstrates, the highest FCR value was found in treatment B (green) at 3.63, while the lowest was in treatment C (blue) at 2.87. Meanwhile for the SR of mahseer, the highest was recorded in treatment B (green) with 100%, C (blue) at 100%, and D (black) at 99.4%, while the lowest was in treatment A (transparent). ANOVA and subsequent Duncan post-hoc test (with a 95% confidence interval) revealed that mahseer reared in variously colored aquariums showed significant difference ($p < 0.05$) in FCR and SGR.

Blood profile of juvenile mahseer

Results from blood tests are presented in Table 2. Parameters tested in blood profile included total number of erythrocytes (red blood cells), total number of leukocytes (white blood cells), hemoglobin concentration, hematocrit value, and blood glucose level. Treatment C (blue) had the highest number of erythrocytes (0.74×10^6 cells/mm^3), while treatment A (transparent) had the lowest (0.40×10^6 cells/mm^3). For leucocytes, treatment A (transparent) had the highest total number of leucocytes (3.05×10^4 cells/mm^2), while the lowest was found in treatment D (black) (2.11×10^4 cells/mm^2).

Each treatment had different hemoglobin concentrations, with treatment C (blue) having the highest (5.45 g/dL) and treatment A (transparent) having the lowest (4.05 g/dL). Further, the highest hematocrit value was found in treatment D (black) at 25.83%, and the lowest was found in treatment A (transparent) at 12.79%. Blood glucose levels also differed in each treatment, with treatment A (transparent) having the highest glucose level (81.00 mg/dL), while treatment C (blue) had the lowest (47.33 mg/dL).

Gross energy and nutritional analysis of masher body

The result of the gross energy or energy retention test in mahseer reared in containers of different colors is presented in Table 3. Each treatment had different gross energy content, where the highest found in treatment A (transparent) with 3,816 cal/g and the lowest found in treatment C (blue) with 3,092 cal/g. ANOVA further revealed that different aquarium colors (as the rearing media for mahseer) showed significant difference in gross energy ($p < 0.05$).

The body nutrition of Mahseer was examined through the amount of moisture, ash, fat, and protein content. Proximate composition analysis was performed on both Mahseer samples

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Table 2. Blood profile of juvenile mahseer reared in variously colored aquariums

Parameters	Aquarium color			
	Transparent	Green	Blue	Black
Total erythrocytes (cells/mm^3)	0.40 × 10^6 ± 0.13	0.58 × 10^6 ± 0.07	0.74 × 10^6 ± 0.15	0.58 × 10^6 ± 0.04
Total leukocytes (cells/mm^3)	3.05 × 10^4 ± 0.64	2.70 × 10^4 ± 0.85	2.25 × 10^4 ± 0.49	2.11 × 10^4 ± 0.49
Hemoglobin (g/dL)	4.05 ± 1.48	4.15 ± 0.07	5.45 ± 0.78	4.65 ± 2.19
Hematocrit (%)	12.79 ± 4.84	12.95 ± 3.44	16.66 ± 1.81	25.83 ± 2.04
Blood glucose (mg/dL)	81.00 ± 1.00	65.00 ± 0.00	47.33 ± 0.58	49.63 ± 0.58

Table 3. Gross energy of juvenile mahseer reared in aquariums of different colors

Aquarium color	Gross energy (cal/g)
Transparent	3,092 ± 16.26^a
Green	3,463 ± 15.55^b
Blue	3,816 ± 65.05^c
Black	3,537 ± 15.55^b

This is data on energy stored in the fish's body.

Table 4. Nutrient analysis of juvenile mahseer bodies in aquariums of different colors

Treatment	Proximate body of fish seeds mahseer			
	Moisture content	Ash	Fat	Protein
Before treatment	72.67 ± 0.16^a	2.49 ± 0.11^b	9.04 ± 0.48^a	12.63 ± 0.24^{ab}
A (transparent)	68.06 ± 0.01^b	1.36 ± 0.16^a	14.34 ± 0.13^b	12.70 ± 0.08^b
B (green)	69.79 ± 0.91^a	2.04 ± 0.14^b	13.47 ± 0.62^b	12.33 ± 0.10^a
C (blue)	66.40 ± 0.06^c	1.95 ± 0.13^{ab}	15.09 ± 0.54^c	13.96 ± 0.06^c
D (black)	66.39 ± 0.26^c	2.22 ± 0.07^b	15.38 ± 0.75^c	13.31 ± 0.33^{bc}

before treatment and samples at the end of the rearing process. Samples before treatment were examined to determine the pre-existing proximate composition to be compared with the post-treatment composition. The results of the nutritional analysis of juvenile mahseers are presented in Table 4. At the beginning of rearing, moisture content in juvenile mahseer exhibited the highest value of 72.67. After rearing, treatments C (blue) and D (black) had the lowest moisture content, with 66.40 and 66.39, respectively. The highest ash content found in the pre-treatment samples was 2.49, while the highest ash content was found in treatment B (black) with 2.22 and the lowest was in treatment A (transparent) with 1.36 after treatment. The lowest fat content in pre-treatment samples was 9.04, while treatment D (black) had the highest fat content (15.38), and treatment C (blue) had the lowest (15.09) after treatment. Finally, protein content in pre-experiment samples was 13.63, while the highest protein content was found in treatment D (black) (13.31) and the lowest was found in treatment B (green) (12.33) after treatment.

Table 5. Water quality of juvenile mahseer cultivation with differently colored aquariums

Parameters	Aquarium color			
	Transparent	Green	Blue	Black
Temperature (℃)	24.6–26.3	24.6–26.2	24.6–26.5	24.7–26.5
Dissolved oxygen (mg/L)	7.50–8.02	7.53–7.87	7.63–7.82	7.76–8.03
Turbidity (NTU)	0–0.01	0–0.02	0–0.04	0–0.03
pH	7.10–7.42	7.10–7.36	7.10–7.57	7.10–7.61
Nitrite (mg/L)	0.043–0.044	0.042–0.046	0.041–0.043	0.041–0.043
Nitrite (mg/L)	4.69–5.02	4.76–4.83	4.63–5.39	4.38–4.80
Alkalinity (mg/L $CaCO_3$)	74.42–85.05	75.92–85.32	75.33–87.48	74.22–85.23
Total Hardness (mg/L $CaCO_3$)	70–80	70–80	68–77	68–80
TAN (mg/L)	0.04–0.05	0.04–0.05	0.04–0.05	0.04–0.05

TAN, total ammonia nitrogen.

Water quality during experiment

Water quality measurements for the 80-days mahseer culture are presented in Table 5. Measurements were carried out regularly to examine water conditions contained in the rearing media. The parameters measured were water temperature, DO, turbidity, pH, nitrite, nitrate, alkalinity, total hardness, and TAN. Water temperature observed during the experimental period in each treatment tended to have an identical average range (24.6℃ to 26.5℃). In the case of oxygen content, treatment A (transparent) had the highest difference in oxygen content (7.50–8.02 mg/L), while treatment C (blue) exhibited the lowest (7.63–7.82 mg/L). Further, the highest turbidity range was found in treatment C (blue) with 0.0–0.04 NTU, while the lowest was in treatment A (transparent) with 0.0–0.01 NTU. Water pH of each treatment tended not to show much difference, where the highest amount ranged from 7.10 to 7.61 in treatment D (black), and the lowest ranged from 7.20 to 7.36 in treatment B (green). Nitrite concentration of all treatments tended to be identical, with 0.041–0.046 mg/L as the general lowest range. The smallest difference between nitrite concentration was found in treatment A (transparent) (0.043–0.044 mg/L), while the biggest was found in treatment B (green) (0.042–0.046 mg/L). Meanwhile for nitrate, treatment C (blue) had the biggest difference in nitrate concentration (4.63–5.39 mg/L) while treatment B (green) had the smallest (4.76–4.83 mg/L). Alkalinity measurements recorded that the smallest to biggest difference in alkalinity level during rearing process ranged from 74.42 to 87.48 mg/L $CaCO_3$. The largest difference in alkalinity level was recorded in treatment C (blue) with 75.33–87.48 mg/L $CaCO_3$, while the smallest was found in treatment B (green) with 75.92–85.32 mg/L $CaCO_3$. Treatments showed not much difference in total hardness levels, with the biggest difference between total hardness level found in treatment D (black) with 68–80 mg/L $CaCO_3$, while the lowest in treatment C (blue) with 68–77 mg/L $CaCO_3$. Furthermore, differences in measurements of TAN parameter tended to be identical for all treatments, ranging from 0.04 to 0.05 mg/L.

Discussion

Growth

The intensification of mahseer cultivation continues to be developed to effectively increase their growth and production effectively. In line with the attempt, the present study intensified mahseer cultivation by manipulating the aquariums' colors to obtain appropriate rearing media backgrounds that is in accordance with their natural habitat. Findings showed that the growth parameters of mahseer were positively influenced by color differences in rearing media. This finding is in line with previous reports where (black) colored tank rearing resulted in the best growth of *Cyprinus* (Jalila et al., 2021), and manipulation of container colors had positive effects on both body and growth performance of fish (Solomon & Ezigbo, 2018). Based on the results of the study, the highest SGR was in the blue container at 2.17%, followed by the black container at 1.83%. The best FCR value was obtained in the color of the blue container (2.87) and the color of the black container (3.03), among all treatments. Meanwhile, the lowest SGR, FCR, and SR values were obtained for the color of the transparent container. This proves that colored containers can increase fish growth compared to transparent containers. This proves that dark-colored containers can increase fish growth compared to light containers. According to several pieces of literature, the responses of different species of fish to the light-color container and dark-color container appear to be governed by changes in energy metabolism and hormone secretion (Ruchin, 2004). Base on the cortisol levels, which are a stress parameter in fish, it shows that the cortisol levels of fish kept in light-colored containers (transparent and green) are higher than those of fish kept in dark-colored containers (blue and black). Accordingly, high levels of cortisol indicate that the fish are experiencing stress. Based on other statements, stress conditions in fish will disrupt the entire endocrine system in the fish's body (Schreck & Tort, 2016). If stress occurs, the body will respond by secreting antioxidant enzymes to suppress stress so that death does not occur. In this condition, the body will use a lot of energy and carry out changes to stop the secretion of growth hormone (Won & Borski, 2013), which causes the growth process to not occur, so that fish kept in dark containers have better growth than fish kept in dark containers.

The results of research on mahseer reared in blue and black containers showed the highest growth performance and The lowest FCR, while the fish reared in transparent and green containers showed the lowest growth and FCR. Acccording to Ninwichian et al. (2018), juvenile guppies (*Poecilia reticulata*), had a higher SGR in blue light (Ruchin, 2004), blue color lightening or background in aquaculture techniques can be used to maximize the growth rates of juvenile tilapia, reduce their stressful behaviors, and minimize their mortality to the lowest rate (Brian, 2015). The present study's findings demonstrated that adjustments to rearing environment for mahseer yielded positive results in terms of body and growth performance. Several characteristics and problems may hinder such performances. Mahseer are surrounded by certain characteristics of their original habitat and are very sensitive to such surrounding environments (Mohapatra et al., 2017). The issue often faced by mahseer culture is the risk of stress instances. Indeed, based on observations before treatment, stress instances in mahseer could cause mass death. The main cause of stress is environmental changes that are intolerable by fish; both physiologically and anatomically.

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Based on parameters examined in this study (growth performance, SR, and FCR), blue and black colored aquariums yielded the best results in mahseer rearing. Blue and black are considered natural as they resemble surrounding colors in mahseer habitat (Solomon & Ezigbo, 2018). Accordingly, mahseer reared in blue and black containers as rearing's background environment responded positively to such colors, which then resulted in better survival. The color of the environment can influence the contrast between food and the background inside the tank, and thus influence its capture and consumption and, consequently, the growth of fish. Several studies have revealed that the rearing tank color influences the growth performance, feed utilization, stress responses, behavior, and skin color of fish (Kesbiç et al., 2016). Color vision requires at least two types of photoreceptors with different spectral sensitivities in blue, green and yellow spectral regions. Different colors have different contrasts against background color and influence the efficiency of detecting and catching the prey or feeds by sight. A high contrast leads to higher visibility and more prey ingestion (Bera et al., 2019). Such positive response can further be confirmed through the positive results of parameters tested in this study including blood profile, gross energy, and nutritional (proximate) analysis, which will be detailed below.

Blood profile

The blood profile of mahseer can be used as an indicator of fish health and as a means of confirming the state of fish body reared in containers with predetermined colors. Blood profile parameters examined in this study included total erythrocytes, total leukocytes, hemoglobin, hematocrit, and blood glucose. Total erythrocytes were the total number of red blood cells contained in the body of mahseer after examination, and total erythrocyte of mahseer in all treatments was sufficient. According to Nainggolan et al. (2021), total erythrocytes for fish are normally 2–300 × 10^5 cells/mm^3. Based on such an account, mahseer in this study had fairly sufficient red blood cells. As the main function of erythrocytes is to carry oxygen and hemoglobin from the gills and lungs to the body's tissues, hemoglobin levels are also strongly influenced by the number of erythrocytes. The higher the number of erythrocytes, the higher the hemoglobin level (Hb).

Normal hemoglobin level in fish ranges between 4.16–5.70 $g.dL^{-1}$ (Fekri et al., 2018). Based on such accounts, treatment C (blue) and D (black) had normal-range hemoglobin level, while treatment A (transparent) and B (green) had below-normal hemoglobin levels. Low hemoglobin level causes disturbances in fish metabolic processes, resulting in energy decline. Lethargic fish tend to stay at the bottom or on the surface of water, leading to a weakened body and reduced appetite. As a result of this reduced appetite, fish will experience immunodeficiency since the body requires food intake in metabolic processes for nutrient absorption which is used as activity and repair of disrupted cells. Environmental stressors, such as inappropriate tank color, may induce changes in the epigenome resulting in life-long impacts on transcriptional regulation (Uren Webster et al., 2018).

Hematocrit is the percentage of red blood cell volumes (erythrocytes), and it is an indicator in measuring erythrocytes level. The normal level of hematocrit in healthy fish ranges from 20% to 30% (Yanuhar et al., 2021). Results presented in this study showed that hematocrit values of treatment C (blue) and D (black) were at the normal range (16%–25%), while treatment A (transparent) and B (green) were below the normal range. A decrease in hematocrit value indicates erythrocyte deficiency which may cause abnormalities and discomfort in the body.

White blood cells (leucocytes) are cells functioning as the defense against abnormalities in the body. The normal number of leukocytes in fish is 20,000–150,000 cells/m^3 (Holladay et al., 2010). As presented in the result section, mahseer in all treatments had total leukocytes above 20,000 cells/m^3 (Table 4). However, a significant difference in leukocytes count between treatment A (transparent) and the rest of the treatments may indicate an increase in the number of leukocytes in the transparent aquarium. Increase in the number of leukocytes may signal abnormalities experienced by fish such as stress and infections (Witeska et al., 2022). The environmentally sensitive mahseer when reared in an environment such as treatment A (transparent) had a high probability of stressing the fish, because a transparent container allows the fish to see movements around it (of either human, animal, or light). Similarly argued that the number of blood cells will quickly change following conditions of disturbances and discomforts experienced by the fish body (Zahangir et al., 2015).

Blood glucose is the next component that plays a very important role, namely as the main fuel supplier in cell metabolism especially brain cells. Glucose is one of stress indicators in fish, according to Nasichah et al. (2016), the normal blood glucose level in fish ranges from 40 to 90 mg/dL. The blood test showed that glucose of mahseer in all treatments was still at the normal level. However, treatment A (transparent) had the highest glucose level compared to other treatments. Hence it can be presumed that there was an increase in glucose level in treatment A (transparent); suggesting that mahseer in this treatment might have experienced stress. Fish that experience stress will have primary and secondary responses, where an increase in glucose is the secondary response following the primary ones (i.e., an increase in stress hormones such as catecholamines and cortisol). Fish experiencing stress will need more energy, which is utilized in the secretion of catecholamine, regulating cortisol, and activating enzymes for the catabolism of proteins. These proteins form and increase amino acids in the blood, activating insulin capable of transporting glucose to normal.

Gross energy

Gross energy is an energy level in the body that can be obtained from feed consumed by the Mahseer fish in appropriate nutrient requirement. Each fish species has similar energy intake and utilization for various activities, except when fish are exposed with environmental (external) or their body internal disorders. The gross energy of fish in treatment C (blue) obtained the highest level, while in treatment A (transparent) obtained the lowest gross energy level. This condition was associated with the blood profiles found in treatment A, which indicates that Mahseer fish in the treatment group are stressed. According to Abdel-Tawwab et al. (2019), fish requires more energy to suppress stress, so the total energy of fish in treatment A has been depleted in a low level, compared to the B (green), D (black), and C (blue) treatments. Therefore, container color background selection is important to prevent stress risk and minimize the energy utilization (Rodgers et al., 2013). A proper habitat may also represent an optimal container color background for growth and physiological condition. Thereby, experiment regarding color preference becomes way more efficient to determine an optimal aquarium color for fish (Li et al., 2016).

Body proximate composition analysis

Body proximate analysis is used to measure crucial contents in the body, such as water, ash, fat, and protein. Such analysis is very important to determine the state of these contents after the fish were cultivated in aquariums of different colors. Body proximate analysis revealed that Mahseer had a normal amount of moisture content before treatment, yet they exhibited a below-normal level of moisture content after treatment (Table 4). Moisture content in fish generally lies between 70% and 80% (Suwandi et al., 2014); hence, moisture content in mahseer fish that has been maintained in clear, green, blue, and black containers was less than that of pre-treatment samples.

High moisture content in fish indicates detached water contents in body tissues. Moisture content in fish greatly affects the meat texture; fish with a normal moisture content tend to have denser meat compared to those with high moisture. The high or low moisture content in fish is affected by the osmotic pressure between body fluids and the environment, where the balance of body substances and the environment involves osmoregulation. Osmoregulation is the regulation of moisture content in the body, where sensory cells monitor ion changes or water balances in the body and the environment (Baldisserotto, 2019). Experimental fish in all treatments had sufficient moisture content, even though it was less than the normal moisture content of fish in general. Therefore, it can be concluded that mahseers reared in transparent, green, blue, and black-colored aquariums did not experience poor physical conditions.

Ash content is an inorganic residue that integrates organic components in a biological Ash content in fish is strongly affected by their habitat and diet, and it is further influenced by the mineral content in their body and habitat. The present study analysis showed that ash content differed between treatments, suggesting that the ash content of a biological material indicates its total mineral. Ash and minerals are typically derived from the biological material itself (indigenous). Ash content in fish is affected by their habitat, which is related to the mineral content in their bodies. Another important parameter is fat content. Fat mainly functions as both a source of formation and reparation for damaged body tissues and as a food reserve; hence, body fat should be kept at a normal level; excess body fat will further cause abnormalities (Pratama et al., 2018). This study's fat proximate analysis showed that each treatment had different levels of fat content, and it exhibited an increase after the experiment. Treatments C (blue) and D (black) had the highest fat content compared to other treatments, indicating that mahseers raised in blue and black aquariums experienced increased appetite; hence, they consumed feed thoroughly and none remained at the base of the aquarium. This finding is in line with the best growth rate obtained by blue and black containers.

Further, protein and fat are connected; they are essential components for growth enhancement in the fish body. Fish protein contains many essential amino acids, which vary greatly depending on the fish type. Quantitatively, proteins mainly function as a source of essential amino acids to synthesize non-essential amino acids and proteins in the body (Hou et al., 2015). Table 4 shows that treatments C (blue) and D (black) had

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higher protein content compared to other treatments, implying that there was a difference in protein absorption among the experimental groups. In each treatment, experimental fish were fed the same type of feed with the same protein content and at the same frequency. Such a discrepancy in protein content may suggest that treatments of certain container colors may resemble habitat-like environments for mahseer. Hence, adjustments to the aquariums (colored with these certain colors) increased fish appetite and did not obstruct the digestion process, so protein absorption was more optimal in treatments C (blue) and D (black) compared to treatments A (transparent) and B (green). Fish experiencing stress need a lot of energy to suppress stress so that the fat and protein content in the fish's body will decrease. If this is related to the cortisol levels in the fish's body, fish kept in green and transparent containers have higher cortisol levels (under stress), which proves that more fat and protein are stored in the bodies of fish that do not experience stress, whereas fish that experience stress have lower levels of fat and protein (Pfalzgraff et al., 2021).

Water quality

In general, water quality parameters during the experiment were considered to be the optimum range for cultivating mahseer (Table 5). The optimal water quality parameters for cultivating mahseer for DO ranges at 6.78–7.71 mg/L, 4.5–5.8 mg/L (Prihadi et al., 2022), pH ranges at 7.0–7.5 (Sudarmaji & Sundari, 2018), water temperature ranges at 21.0℃–25.0℃, 22.1℃–27.3℃ (Subagja et al., 2021), nitrite at 0.016–0.022 mg/L, nitrate at 0.71–1.40 mg/L, and alkalinity at 84.8–86.8 mg/L (Radona et al., 2017). Water quality is one of the most important parameters in examining water conditions, and water quality management must be carried out to support the success of cultivation, which subsequently increases production. Findings showed that colored containers did not have any adverse effects on overall water quality during cultivation.

Water quality decline is caused by several factors, namely the increase in ammoniac content (TAN), extreme pH content, low DO content, and fluctuating temperature (a drastic increase or decrease in temperature). Water quality parameters are indirectly interrelated (Pratama et al., 2022). For instance, fluctuating temperatures will cause digestive disorders where the digestion process slows down and results in a decreased appetite. In turn, decreasing appetite can increase the amount of uneaten feed lying at the base of the container, which will then be mixed with fish feces, increasing ammonia content. An increase in ammoniac content affects fish growth through reduced oxygen and increased carbon dioxide (CO_2), where fish will instantly experience oxygen deficiency, disturbing the function of the body organs and then damaging body tissues (Pratama et al., 2022). Thus, water quality management is very essential to reduce residual metabolic waste, which may turn into harmful substances like ammonia. Optimal water quality parameters allow for a satisfactory cultivation system, while poor water quality may accumulate harmful substances such as toxins or toxic substances derived from the food waste lying at the base of the container, causing diseases in fish (Asaduzzaman et al., 2018).

Competing interests

No potential conflict of interest relevant to this article was reported.

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Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

Research ethic clearance supplied by Fish Health Laboratory IPB University (Bahasa Indonesia).

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