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THE EFFECT OF FEEDING BLACK SOLDIER FLY LARVAE WITH DIFFERENT TYPES OF FEED ON THE CHROMATOPHORE CELL RESPONSE IN KOI FISH *(Cyprinus carpio)*

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ABSTRACT:

This study aimed to analyze the optimal type of BSFL feed based on carotenoid content testing and determine the BSFL feed type that can enhance the color quality and chromatophore cell response in Koi fish. The study employed an experimental approach with a completely randomized design (CRD) comprising 4 treatments and 3 replications: A - Commercial Feed, B - BSFL fed with pumpkin, C - BSFL fed with carrots, and D - BSFL fed with tomatoes. Koi fish with a length of 5-6 cm were used for the experiment. The results showed that feeding BSFL with carrots produced the highest carotenoid content in BSFL, at 1.1631 µg/g. This treatment also significantly impacted ($P<0.05$) the color quality of the fish, scoring 25.08 on the Toca Colour Finder scale. The treatment of feeding BSFL with carrots also resulted in the highest number of chromatophore cells, with 3491 cells. Therefore, it is recommended to feed Koi fish with BSFL fed with carrots, as they have a high carotenoid content and can effectively enhance the color quality and chromatophore cells of Koi fish.

Keywords: BSFL Feed, Carotenoids, Koi Fish, Color Quality, Chromatophore Cells.

1. INTRODUCTION

Koi fish (Cyprinus carpio) are ornamental fish highly valued for their vibrant colors and body shapes, making them very popular. The color is one of the main reasons these ornamental fish are so sought after. The pattern and color changes in koi fish are influenced by growth, genetics, and environmental factors such as air temperature, water quality, sunlight, and diet (Li et al., 2023). According to Sholichin et al. (2012), the color of koi fish is influenced by the presence of chromatophores in the fish's epidermis layer. Chromatophores are classified into five categories: erythrophores produce red and orange colors, xanthophores produce yellow, melanophores produce black, leucophores produce white, and iridophores reflect light.

Efforts to enhance the color beauty of fish can be made by adding pigment sources to their diet (Sukarman et al., 2018). Adding color-enhancing sources to the diet can boost the fish's body color or help maintain the existing colors. The quality of ornamental fish colors can be improved by providing food containing carotenoids. According to García et al. (2013), carotenoids are a primary source that triggers the pigmentation process in ornamental fish. Carotenoids are natural pigments that can contribute to yellow, orange, purple, blue, and green colors in both plant and animal foods (Nakano & Wiegertjes, 2020). Sources of carotenoids can come from organic materials such as carrots, sweet potatoes, pumpkins, spinach, corn, and various green vegetables.

Using carotenoids as a pigment source in fish will be more effective if these substances are within a living organism. According to Wagde et al. (2018), the carotenoid content in yellow pumpkin ranges from 29.16 mg/100g to 154.76 mg/100g of β-carotene (Norshazila et al., 2014). Research by Saiful (2017) showed that the higher the dose of yellow pumpkin given in the diet, the greater the brightness of the koi fish scales. The increased brightness in treatment C (40%) compared to other treatments was due to the higher pigment content in the diet, attributed to the higher dose of yellow pumpkin flour.

Carrots are a natural source of beta-carotene that can enhance the quality and brightness of ornamental fish colors, such as koi fish. Carrots (Daucus carrota) are one of the ingredients that produce carotene, which can beautify the color of ornamental fish. Carrots also contain carotenoids, which can enhance fish color similarly to spirulina (Sunarno, 2012). Carrots have a carotenoid content of 123.07 mg β-carotene/100 g (Yuni, 2022). Research by Sihaloho et al. (2023) showed that adding carrot flour to koi fish feed positively affected growth and color brightness. Tomatoes are a fruit with high red pigment content and are relatively inexpensive, making them good subjects for further research. The carotenoid content in ripe tomatoes is 113.05 mg/100 g (Novita et al., 2015).

Providing carotenoids from live feed can improve fish color quality. One live feed that can serve as a carrier of carotenoid pigments is the Black Soldier Fly larvae. Black Soldier Fly larvae (Hermetia illucens) are an alternative feed that meets the requirements as a protein source, categorized as a significant protein source that can replace fish meal up to 50% without adverse effects on fish (Jeyaprakashsabari & Aanand, 2021). Black Soldier Fly larvae are a natural feed type with high protein content, containing 37-62% crude protein, 21-38.6% lipids, 7-39% fat, 9-28% ash, and 4.8-5.1% calcium (Barragan-Fonseca et al., 2017), and have beneficial effects for enhancing fish immunity (Wang & Shelomi, 2017).

Additionally, Black Soldier Fly larvae have an organ called trophocytes that store nutrients from their feed (Liu et al., 2018). The growth medium or diet for BSFL can affect the quality of the larvae produced. High-quality and sufficient substrates are necessary for good larval development (Hem et al., 2008). Black Soldier Fly larvae can be used to convert waste such as agricultural, livestock, and other organic wastes (Rana et al., 2015). In koi fish farming, color and optimal growth are crucial factors, as vibrant and beautiful colors are highly valued in the ornamental fish market. Feeding koi with Black Soldier Fly larvae has been extensively studied, focusing on growth and as a substitute for fish meal in fish pellet production (Daniel et al., 2018). Besides being an alternative protein source, Black Soldier Fly larvae can be given fresh to fish as an alternative feed. Trials with ornamental and carnivorous fish have shown that species like arowana, betutu, catfish, and snakehead fish favor fresh Black Soldier Fly larvae (Fahmi et al., 2020).

Providing Black Soldier Fly larvae as koi fish food does not only depend on the type of food but also on the cultivation media or diet used to develop the larvae. Therefore, further research is needed to analyze the effect of giving Black Soldier Fly larvae Using different types of feed on the response of koi fish chromatophore cells.

2. MATERIALS AND METHODS

Research Design

This study explores the effects of feeding Koi fish with BSFL reared on different diets. Using a completely randomized design (CRD), there are four treatment groups, each replicated three times. The treatment groups are:

A. Commercial feed (Control)
B. BSFL fed with Pumpkin
C. BSFL fed with Carrot
D. BSFL fed with Tomato

The BSFL, after being reared on the specified diets, are then fed to the Koi fish. The fish are fed twice daily at 08:00 and 16:00, with feed provided ad libitum, meaning they are given as much feed as they can consume until they are no longer responsive to it.

Preparation of Test Feed

The process of cultivating BSFL for Koi fish feed begins with setting up 60x60x10 cm containers. Organic feeds such as pumpkin, carrot, and tomato are blended and placed into these containers. BSF eggs from a breeder in Makassar are evenly distributed over the organic feed to ensure even growth. The eggs hatch into BSFL after about three days. Throughout their growth period, the BSFL are given adequate feed and maintained under optimal environmental conditions. After 10 days, the BSFL are ready to be harvested and used as Koi fish feed.

Preparation of Test Animals

Koi fish maintenance starts with preparing 50x50x30 cm tanks, each equipped with aeration systems connected to a blower for oxygen supply. Sixty Koi fish, aged two months and about 5-6 cm long, are used. These fish, initially dull in color with a score of 8 on the Toca Colour Finder (TCF) scale, are distributed among the tanks assigned to treatments A, B, C, and D, with each tank housing 5 fish. The Koi are then fed with pellets and the prepared BSFL during the study period.

Research Parameters

Carotenoid Content Analysis in BSFL

Carotenoid content analysis is performed on BSFL reared on diets of pumpkin, carrot, and tomato.

Koi Fish Color Observation

Based on Diler and Dilek (2020), the development and changes in the observable color of Koi fish are recorded descriptively, with brightness scores illustrated in the following figure.

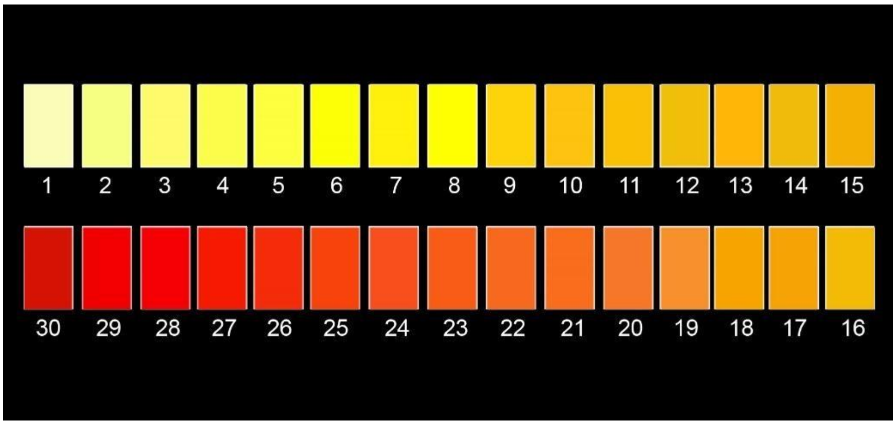


Figure 1. TCF Scale Color Observations.

Figure 1 shows a color scale numbered from 1 to 30. The upper part consists of 14 yellow boxes with different intensities, ranging from light yellow to dark yellow, each numbered sequentially from 1 (lightest yellow) to 15 (darkest yellow). The lower part displays a different color scale with 15 boxes, transitioning from dark yellow through orange to dark red, also numbered sequentially from 16 (dark yellow) to 30 (dark red).

Chromatophore Cell Observation

The observation of chromatophore cells is performed with a microscope at 100x and 400x magnifications. The count of chromatophore cells is conducted at the start and conclusion of the study using the Image J software. The specific target for cell counting is the dermis layer, focusing on the lower portion of the dorsal fin, which is prepared as a sample (Aras, 2015).

Survival Rate

The survival rate depends on multiple factors such as water quality, food supply, presence of predators, diseases, and management practices for the aquatic environment (Wiley-Blackwell, 2012). The SR is calculated using Effendi's (2002) formula:

$$SR = N_t/N_0 \times 100\%$$

Explanation:
SR = Survival Rate (%)
Nt = Total number of fish at the end of the study
No = Initial number of fish at the beginning of the study

Water Quality

The water quality parameters measured in this study are temperature, dissolved oxygen (DO), pH, and ammonia. DO and pH are measured once a week, while temperature is measured daily. Ammonia is measured at the beginning and end of the study.

Data Analysis

The data collected includes the carotenoid content of BSFL, Koi fish color, Koi fish chromatophore cells, Koi fish survival rate, and the physical and chemical properties of the water. Observations of color, the number of chromatophore cells, and Koi fish survival rate were analyzed using analysis of variance (ANOVA), and for data showing significant effects, further testing was carried out with the W-Tukey post hoc test to determine differences between treatments. The Tukey HSD test was used for comparing the differences between treatments. Statistical tests were conducted using SPSS software version 26.0. The data on BSFL carotenoid content, Koi fish chromatophore cells, and water quality were analyzed descriptively, comparing the results obtained between treatments.

3. RESULTS AND DISCUSSION

Carotenoid Content in BSFL

The results of carotenoid testing in BSFL fed different diets such as pumpkin, carrot, and tomato for 10 days of cultivation are presented in Table 1.

Table 1. Carotenoid Content in BSFL Fed Different Diets

No.	Sample Code	Parameter
		Carotenoid content (µg/g)
1.	BSFL fed with Pumpkin	0.2987
2.	BSFL fed with Carrot	1.1631
3.	BSFL fed with Tomato	0.9384

Based on Table 1, there is a difference in carotenoid content in BSFL after being fed different diets.

Based on the carotenoid content test results of BSFL, as shown in Table 1, it was found that the combination of BSFL with carrots resulted in the highest carotenoid content compared to other feed combinations. The carotenoid content in treatment C, where BSFL were fed carrots, reached 1.1631 µg/g, indicating the substantial contribution of carrots as a good source of carotenoids. In contrast, treatment B, where BSFL were fed pumpkin, yielded a relatively low carotenoid content, only reaching 0.2987 µg/g. Treatment D, where BSFL were fed tomatoes, resulted in a slightly higher carotenoid content in BSFL compared to pumpkin, at 0.9384 µg/g. These results suggest that carrots have great potential as an additional feed to enhance the carotenoid content in BSFL, which could be an important consideration in developing higher-quality feeds.

Based on the morphological observations of the body color of BSFL in each treatment, it was found that the colors varied. The body color of BSFL in treatment (B), fed with pumpkin, appeared white to yellowish. Treatment (C), fed with carrots, resulted in a reddish-orange body color, while treatment (D), fed with tomatoes, resulted in a light pink body color. This variation is believed to be due to the influence of the feed treatments, which affect the body color pigmentation of the BSFL. This is in line with Prasetyo and Kataren (2015), who stated that an animal's body color is related to the pigment cells inside the animal's body, which can be influenced by the presence of pigment substances (natural dyes) such as carotenoids or β-carotene and xanthophylls in food, which are then synthesized by the animal's body that consumes them. Pumpkin, carrot, and tomato all contain β-carotene. This is consistent with the statement by Gull et al. (2015) that the presence of β-carotene in vegetables such as carrots or fruits containing carotenoid compounds will provide coloring effects such as yellow, orange, and reddish colors that naturally occur.

Color of Koi Fish

The research results on the color brightness of Koi fish during a 60-day rearing period show an improvement in color, including the control group. The color enhancement in Koi fish can be seen in Table 2.

Table 2. Observation Results of Color Changes in Koi Fish

Parameter	Observation Results of Color Changes (TCF)

Color of Koi Fish	A	B	C	D
	20.33±0.305[a]	21.55±0.180[b]	25.08+0.381[d]	22.80±0.346[c]

Note: a, b, c, and d = letter notation indicates a significant difference at the 5% level in the W-Tuckey test.

The observation results of color changes in Koi fish fed with different types of feeds are presented in Table 2. Based on Table 2, it can be seen that the highest color change value in Koi fish was in treatment C, where BSFL were fed with carrots, while the lowest was in treatment A, commercial feed. The analysis of color change data showed a significant difference. It is suggested that Koi fish can utilize the carotenoids in the feed, which are absorbed and can change the color of the Koi fish compared to the control treatment fed with pellets. The color of the fish is the most important factor in determining the economic value in the ornamental fish industry. The main factors determining the economic value of ornamental fish include pigmentation, fin, and tail shape (Haridas et al., 2019). Several factors that can lead to a decrease in fish color include stress, water quality, physiological status, etc. The lack of a carotenoid source in fish feed will result in color fading in fish (Zutshi and Madiyappa, 2020).

The second figure illustrates that every type of feed given to Koi fish is metabolizable and can be stored within their pigment cells. The results indicate that the group fed BSFL with carrot (treatment C) exhibited a marked improvement in color intensity compared to the group receiving commercial feed (treatment A).

The use of BSFL fed with carrots (C) showed different results compared to treatments A (score 20), B (score 22), and C (23). In treatment C, BSFL fed with carrots and then applied as feed for Koi fish will affect the absorption of carotenoids in the fish's body. The testing of carotenoid feed resulted in treatment C having 1.1631 µg/g, as shown in Table 1. This is supported by (Hadijah et al., 2020), where differences in the brightness level of fish colors occur due to differences in the amount of carotenoids contained in the feed provided.

The carotenoids contained in BSFL can increase the activity of enzymes involved in the formation of erythrophore pigment cells. The high protein content in BSFL can also increase cell division activity, stimulating the production of MSH hormone, which can increase the movement of pigment granules in chromatophores. Pigment granules in chromatophores will be dispersed into the cells, allowing the cells to absorb light perfectly, thus increasing the color intensity of the fish scales (Hutabarat, 2019). According to Pratama (2018), there are two factors that affect the color quality of fish, namely internal and external factors. Internal factors are factors that come from within the fish's body that are constant, namely genetics. Meanwhile, external factors are factors that come from outside the fish's body, namely the fish maintenance media.

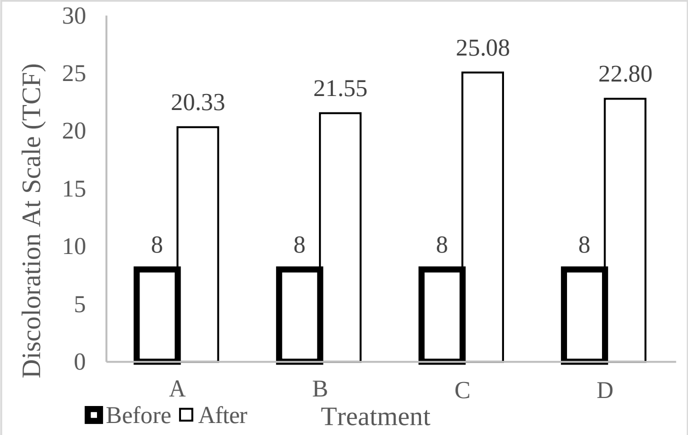
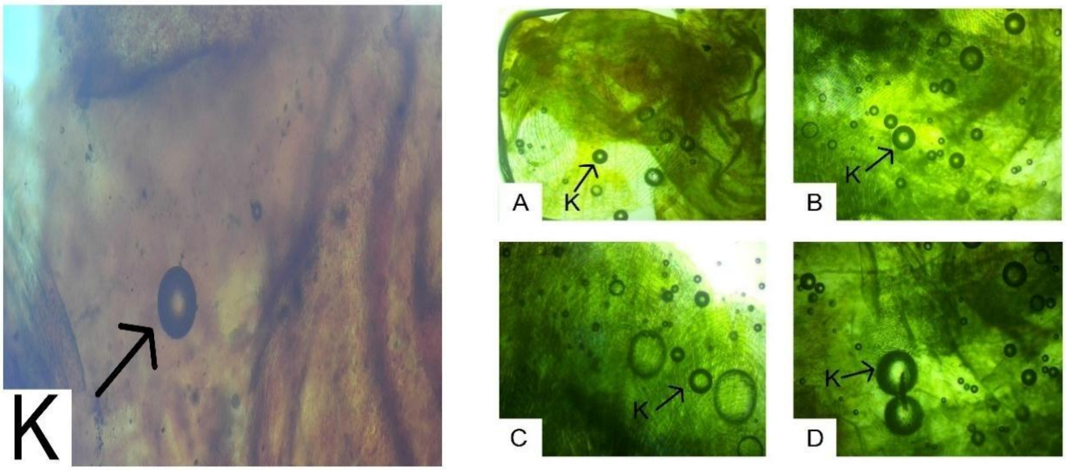


Figure 2. Color Changes in Koi Fish (Cyprinus carpio) After 60 Days of Rearing.

Koi Fish *(Cyprinus carpio)* Chromatophore Cells

The results of observing the chromatophore cells of koi fish using a microscope with 100x and 400x lenses for each treatment during the study can be seen in Figure 3.



Note: Symbol (K) represents a chromatophore cell.

Figure 3. Illustrates the changes in chromatophore cells in koi fish from the beginning to the end of the study.

Chromatophore cells are round pigment cells located in the fish's skin layer (Umalekhay et al., 2020). Microscopic observations of chromatophore cells revealed differences between the initial chromatophores (before treatment) and after treatments A (commercial feed), B (BSFL fed pumpkin), C (BSFL fed carrot), and D (BSFL fed tomato). The distribution of chromatophore cells in koi fish under the control treatment (A) was relatively sparse compared to those treated with BSFL fed different feeds. In the treatment without BSFL, the distribution of chromatophore cells in koi fish was more centralized at a single point, resulting in suboptimal light absorption. In contrast, treatments with BSFL fed different feeds showed a more dispersed distribution of chromatophore cells, leading to optimal light absorption and thus enhancing the color performance of the fish. This is supported by Subamia et al. (2013), who stated that the movement of pigment granules in chromatophores can be classified into two types: chromatophores with pigment granules clustered near the nucleus and chromatophores with granules spread throughout the cell. Pigment granules dispersed within the cell allow for perfect light absorption, resulting in brighter and more distinct fish scales. According to Widinata et al. (2016), physiological color change is caused by the activity of pigment granules or chromatophores moving within the cells.

The observations of chromatophore cells under the microscope were further quantified using the Image J application to facilitate counting. The ANOVA test revealed a significant difference ($p < 0.05$) in the number of chromatophore cells across the various feed treatments. According to the W-Tukey post hoc test, the BSFL fed with carrot and those fed with tomato produced the highest number of chromatophore cells, and there was no significant difference between these two treatments. Initially, before treatment, there were 213 chromatophore cells. After administering different feeds, an increase in the number of chromatophore cells was observed. Treatment A with commercial feed (control) showed the lowest number of chromatophore cells, totaling 619 cells. The number of chromatophore cells continued to increase in treatment B with BSFL fed pumpkin, reaching 1,884 cells; treatment C with BSFL fed carrots, reaching 3,491 cells; and treatment D with BSFL fed tomatoes, reaching 2,624 cells. The highest increase in color intensity, as seen from the number and distribution of chromatophore cells, was observed in treatment C, where BSFL fed with carrot was applied to the Koi fish. The more abundant and dispersed the chromatophore cells, the better the resulting color (Virgiawan et al., 2020). This is likely due to the influence of feeding BSFL with carrot, which serves as a source of carotenoids in the test feed, thus enhancing color pigmentation in the fish's body.

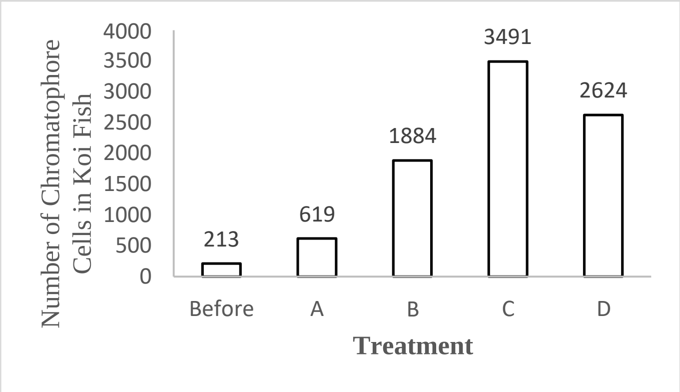


Figure 4. Initial and Final Number of Chromatophore Cells in Koi Fish (Cyprinus carpio).

Survival Rate

Survival rate is the comparison of the number of living fish from the beginning to the end of the study, expressed as a percentage of the total number of fish. It represents the number of organisms alive at the end of the maintenance period, expressed as a percentage (Rikawati, 2018). The observation results of Koi fish (Cyprinus carpio) Survival Rate over a 60-day maintenance period with different feed treatments are shown in Figure 5. It can be seen that the survival rate of Koi fish in treatment A was 86.7%, treatment B was 86.7%, treatment C was 93.3%, and treatment D was 100%. The ANOVA results indicate a value of 0.627 (Significance Value $P>0.05$), suggesting that there is no significant difference between treatments (A, B, C, and D) regarding the survival rate of Koi fish.

The observation results indicate that in treatment D, where BSFL fed with tomato were used, the survival rate (SR) of Koi fish reached 100%, meaning that there were no fish deaths during the study. This result differs from other treatments where fish mortality was observed, although statistically, there was no significant difference. The high SR in treatment D is believed to be due to several factors. Feeding BSFL with tomato before providing them as fish feed can enhance the nutritional quality of the BSFL. Tomatoes are rich in vitamins, minerals, fiber, and antioxidants like lycopene, which are beneficial for health. This complete nutrient intake can improve the nutritional value of BSFL as Koi fish feed, and the better nutrition from BSFL fed with tomato can enhance the immune system of Koi fish. Antioxidants from tomatoes, such as lycopene, play a role in protecting fish from oxidative stress and improving overall health, ensuring no fish deaths during the experiment. Other compounds in tomatoes may also contribute to maintaining the fish's optimal condition (Nguyen et al., 2020).

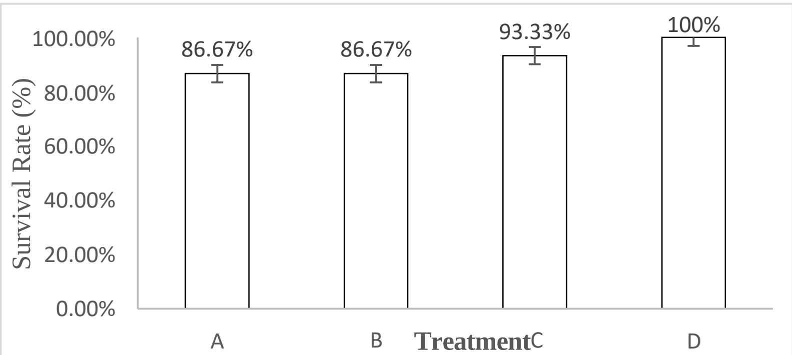


Figure 5. Survival Rate of Koi Fish (Cyprinus carpio).

Water Quality

The findings related to water quality parameters over the 60-day maintenance period are presented in Table 3.

Table 3. Water Quality Measurement Results.

Based on the temperature measurements in the Koi fish maintenance media during the study, it ranged from 28 to 30 °C. This range is still optimum for the life of Koi fish according to the SNI 2017 standard. This can be supported by Putri et al. (2020), stating that Koi fish can still survive in water temperatures of 25 - 35 °C. Temperature is an important factor that plays a role in supporting the growth, color quality, and survival of fish.

The pH value in the Koi fish maintenance media during the study ranged from 6.7 to 7.1. This pH range is considered optimal for the life and growth of Koi fish. This is supported by Sutiana et al. (2017), stating that the optimal pH range for the growth of Koi fish is between 6.5 - 8.5. The pH value indicates whether the water is acidic, basic, or neutral. pH determines the chemical processes in water because pH that is too acidic or basic can cause fish to become passive in movement, as fish are less adaptable to dirty water, resulting in pale-colored fish.

The dissolved oxygen (DO) value in the Koi fish maintenance media during the study ranged from 3.14 to 7.5 mg/L. This DO value is still within the optimal range for the life of Koi fish. This is supported by Widinata et al. (2016), stating that the optimum dissolved oxygen level in water for the growth of Koi fish is more than 5 mg/L. The higher the DO value in water, the better the water quality for fish maintenance.

The range of ammonia during the study was 0.0009 - 0.0092 mg/L, which is less than 1 mg/L. This range is still within the tolerance limits of Koi fish, according to Hastuti and Subandiyono (2011).

4. CONCLUSION

Time	Water Quality	Value	Standard Value (SNI) 77334, 2017
Every day	Temperature °C	28-30	25-30
Once a week	pH	6,7-7,1	6,5-8
Once a week	DO (mg/L)	3,14-7,5	>3
Beginning and End	Ammonia (mg/L)	0,0009-0,0092	<1

Based on the 60-day study, BSFL fed with carrot is an alternative BSFL feed that has the highest carotenoid content compared to other treatments. It is recommended to feed Koi fish with BSFL fed with carrot, as it has a high carotenoid content and can stimulate the color quality and chromatophore cell response of Koi fish effectively.

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