

THE EFFECT OF *SPIRULINA* POWDER ON THE FEED CAN GIVE EFFECT BRIGHTNESS COLOR GUPPY FISH (*Poecilia reticulata*)

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Abstract

Guppy fish (*Poecilia reticulata*) is one of popular ornamental fish because of their unique color patterns, the color of ornamental fish will fade if their feed does not contain enough pigmentation to keep them bright. This study aimed to determine the effect of adding *Spirulina sp.* powder with different dosage to the feed on the color brightness of guppy fish. This study used a completely randomized design (CRD) with five treatments (feed added with *Spirulina sp.* powder as much as 0%, 0.3%, 0.6%, 0.9%, and 1.2%) and three replications. The test fish were male HB Red guppy fish with a size of 3 cm and a density of 3 fish/aquarium. Water changes (siphoning) was done every morning, and feeding was done twice a day. The parameters observed in this study were the chroma value, survival rate, and water quality. The result showed that the effect of adding *Spirulina sp.* powder to the feed at a dose of 0.9% and 1.2% had a significant effect on the color brightness. The survival rate indicates that the outcomes were not significantly different between treatments from a 100% success rate. The adding of *Spirulina sp.* powder increases the brightness of Guppy fish. The brightness color is very important in the Guppy fish farming, because color is the main indicator in ornamental fish. The water has a good quality, with an average temperature of 25.8-26°C and a pH of 7.9-8.0.

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Introduction

Ornamental fish farming has the potential to be developed in Indonesia and has a high market opportunity, both domestically and abroad. According to Anwar *et al.*, (2021), Indonesian ornamental fish products come in a variety of species, including freshwater ornamental fish and marine ornamental fish (Gani *et al.*, 2021; Nurjirana & Keith., 2022; Hasan *et al.*,

2022). One of the most popular freshwater ornamental fish is guppy fish. Guppy fish which is also widely known as million fish or rainbow fish, is a fish that widely distributed in various countries, especially in tropical regions (Panjaitan *et al.*, 2016). The problems faced by fish cultivators including guppy fish are related to the feeding process to increase growth (Afifah *et al.*, 2020; Cahyani *et al.*, 2021; Kurniawan *et al.*,

2018), fish health (Soedibya *et al.*, 2021) and increased fish brightness to make fish more attractive. Feed is an important media for manipulating ornamental fish cultivation technology to further develop.

Guppy fish is one of the ornamental fish that is in great demand because it has interesting color variations such as red, blue, yellow, and other colors, especially in male fish. The shape of the tail varies, for example, fan-tailed, rounded, or wide Pratama *et al.*, (2018). To produce the best colors, ornamental fish require a good environment and foods with high nutrients. Foods with high nutrients will support good color, health, and seed quality (Anwar *et al.*, 2021).

Color is one of the reasons why ornamental fish are in demand by the public or ornamental fish enthusiasts. During maintenance, ornamental fish will fade if their feed does not contain enough pigmentation to keep them bright. This is because fish cannot synthesize or produce color pigments in their bodies (Kurnia *et al.*, 2013). Therefore, color pigments must be added to the feed to maintain and improve the color performance of ornamental fish. Currently, cultivators often apply synthetic dyes in feed, such as synthetic astaxanthin and lycantin to increase color brightness in

ornamental fish. For this reason, it is necessary to find an alternative to additional supplements that utilize natural ingredients, one of which is using microalgae (Rosid *et al.*, 2019).

Spirulina sp. is one of the microalgae that contains color pigments, the dyes are known as carotenoids (Wicaksono *et al.*, 2019; Budi *et al.*, 2020; Satyantini *et al.*, 2022). Carotenoids are substances used for skin coloring in fish. The effect of the presence of carotenoids is that it can cause an increase in the brightness of the red color in fish. *Spirulina* contains phycocyanin, chlorophyll-a, and carotene. Carotene is composed of xanthophyll (37%), β -carotene (28%) and zeaxanthin (17%). The carotenoid content in *S. platensis* can also be an antioxidant and used as a food supplement (Nafsihi & Hidaidah, 2016).

The effect of adding *Spirulina sp.* powder to the feed has been studied in goldfish (Noviyanti & Maharani, 2015) and Sumatran fish (Nafsihi & Hidaidah, 2016). In both studies, the best concentration in increasing the brightness of fish color was 1.2% with treatments of 0%, 0.3%, 0.6%, 0.9%, and 1.2%, respectively. The results showed that *Spirulina* powder increased color brightness in goldfish and Sumatran fish. This study choose guppy fish because

they are very popular ornamental fish species with high selling prices. So far, there has been no research on the addition of *Spirulina sp.* powder in feed on the color brightness of guppies.

The purpose of this study was to determine the effect of adding *Spirulina sp.* powder to the feed on the color brightness of guppy fish and know the best dosage of *Spirulina sp.* for increasing the brightness of the color of guppy fish. This research is expected to provide scientific information for the academic community as a source for further research regarding the effect of adding *Spirulina sp.* to the feed increases color brightness in guppies. This information can be used as guidelines and further studies to increase the productivity of guppy fish cultivation.

Materials and Methods

This research was conducted from March to April 2021 at the Pescica Marina Laboratory, Fisheries and Marine Sciences Faculty, Jenderal Soedirman University, Purwokerto, Central Java. The object used in this study was guppy fish strain HB Red. The fish used were two months old with an average weight of 0.3 g and a total length of 3 cm. The tools used for maintenance were 15 aquarium units (20×10×15 cm) and supplied with aerators aerator. The tools

used to measure water quality were pH meter (Hanna instruments) and dissolved oxygen meter (Lutron DO-5510). Other tools used were HP (Oppo A-1k), analytical scale (OEM g-max 500 g), mini photo box, and camera (Canon EOS 100D). The materials used in this study were progol, *Spirulina sp.* powder, and feed. Progol functions as an adhesive for fish feed. *Spirulina sp.* serves as a source of carotenoids. The feed serves as a basic ingredient for fish feed with a composition of 46% Protein, 6% Fat, 5% Fiber, and 8% Ash.

Research Procedure

Maintenance Container Preparation

The rearing container used in this study was an aquarium with a size of 20 x 10 x 15 cm as many as 15 units. Each aquarium cleaned and then dried for 15 minutes. Each aquarium given aeration that has been adjusted to the strength of the oxygen supply. Then, the aquarium filled with two liters of water.

Feed Preparation

The feed used during the study was feed (Protein 46%, Fat 6%, Fiber 5%, Ash 8%) and *Spirulina sp.* powder. The stages of mixing *Spirulina sp.* in the feed are: *Spirulina sp.* powder mixed with progol (5 kg⁻¹ feed) in one container and stirred until

evenly distributed. Then, *Spirulina sp.* powder which has been stirred evenly with progol is given water at a dose of 125 ml Kg⁻¹ of feed and left for 10 minutes. Next, the feed was poured into the *Spirulina sp.* powder container which had already mixed with progol. Then, stir until all the *Spirulina sp.* powder evenly on the feed. If all of the *Spirulina sp.* powder is evenly distributed, then the mixture is dried and left to dry for 30-60 minutes. During drying, if the feed changes in color and smell, it must be discarded and remade. Feeding was done regularly.

Fish Culturing or Pisciculture

The fish were first acclimated for three days in a holding tank before they were stocked in rearing containers. The process of acclimatization aims for fish to adapt to the new environment and the type of feed given. The maintenance of this fish conducted for three days in a holding tank. After that, sorting is done to select healthy fish and do not have wounds. Next, the fish were stocked in the aquarium. Guppy fish rearing conducted for 30 days at the Pescica Marina Laboratory. The guppies were fed twice a day at 8 a.m. and 4 p.m. Local time. Water changes are conducted through the siphoning process. Siphoning was done every day, namely the morning before

feeding. Siphoning aimed to remove the rest of the feed and feces in the aquarium, so there is no buildup and decay in the media.

Research Design

This research was conducted with a completely randomized design (CRD) consisting of 5 treatments with 3 replications, so it became 15 experimental units. The experiments carried out are as follows:

T1: Feed without the addition of *Spirulina sp.* powder (Control).

T2: Feed added with *Spirulina sp.* powder 0.3%.

T3: Feed added with *Spirulina sp.* powder 0.6%.

T4: feed added with *Spirulina sp.* powder 0.9%.

T5: Feed added with *Spirulina sp.* powder 1.2%.

Data Collection

Color Brightness Value (Chroma)

Color intensity data collection from guppies was conducted once every 10 days during maintenance. Determination of the color intensity value in guppies using the colorimeter application, this application uses the value or number of Light (light or dark), a*, b*, and chroma. Chroma is the color intensity that distinguishes strong colors from weak colors based on the original

pigment (Nurmawati, 2011). This color determination is based on the 1976 CIE standard, based on the CIE L* a* b* (CIE LAB) color system with parameters lightness (L*), redness (a*), yellowness (b*) (Hasbullah & Umiyati, 2017). The L* value indicates light and dark (Joiner, 2004). A positive (a*) value indicates red; gray if the value is 0; (a*) negative indicates green; a positive (b*) value indicates yellow; gray if the value is 0; (b*) negative indicates blue; the value (L*) ranges from 0 (black) to 100 (white); the value of C indicates the brightness of the color from low (faded) to high (deep). The value used is the chroma value. If the chroma value increases, the color will be lighter, while if the chroma value decreases, the color will fade.

Data retrieval conducted by taking pictures of fish using a DSLR camera (Canon EOS 100D) and a mini studio box with a position parallel to the object, then the photo will be exported in the Colorimeter application. Fish color data measured is the tail with the dominant color. Then the photos are exported to Handphone (Oppo A-1k) in the Colorimeter application and matched against the colors contained in the 1976 CIE standard. The chroma value listed on the Colorimeter application obtained on the first day is recorded and

then compared with the color data for each 10-day color measurement schedule and the collected data were analyzed by Anava analysis.

Fish Survival Rate (SR)

Fish survival is the ratio of the number of live fish to the number of fish stocked at the beginning of the study. Calculating survival using the formula (1) of Rosid *et al.*, (2019):

$$\text{Survival rate (\%)} = \frac{N_t}{N_o} \times 100 \dots\dots\dots (1)$$

Where:

SR : Life sustainability (%)

Nt : Number of live fish at the end of the study (fish)

No : Total number of fish at the beginning of stocking (fish)

Water Quality

Water quality is very influential on the survival of fish and color brightness in ornamental fish. The water quality observed at the time of the study for the supporting materials were temperature and pH.

Data Analysis

The data obtained from the research results were processed using archsin transformation and analysis of variance with the F test (ANOVA) to determine whether or not there was an effect of giving *Spirulina sp.* powder in artificial feed on the color

Table 1. Water quality parameters

Parameters	Range	Quality Standards *
Temperature	25.8 - 26 ⁰ C	25 – 32 ⁰ C
pH	7.9 – 8.0	6 – 9

brightness of guppies. If there is a difference between treatments, then proceed with the BNT test at a 95% confidence level. While the water quality is analyzed descriptively.

Results

The results of statistical analysis regarding the effect of adding *Spirulina sp.* powder to the feed on color brightness of guppies reared for 30 days.

Water Quality

Water quality is one of the supporting factors. Water quality measurements are measured every day. The parameters observed were temperature and pH. The water quality parameters measured during the study did not show significantly different numbers and all the water quality parameters measured were within the quality standard for ornamental fish maintenance. Measurement of water quality during the study can be presented in Table 1.

Color Brightness Value (Chroma)

Based on statistical analysis, it concludes that the addition of *Spirulina sp.*

to the feed was significantly different (T<0.05) between testing, so a further test of BNT conducted at a 95% confidence level resulting in T1 and T2 not being significantly different, but significantly different from T3, T4, and T5. T2 and T3 were not significantly different, but significantly different from T4 and T5. The measurement results of the chroma value can be seen in Figure 1.

T3 with T4 and T5 were not significantly different. T4 and T5 were not significantly different. So after it can be concluded that, the addition of *Spirulina sp.* powder. can increase the brightness of fish color, and the best dose to increase the brightness of fish color in treatment 4 (0.9%) and treatment 5 (1.2%). According to (Malinde *et al.*, 2018) fish color changes depending on the amount of color composition in the feed. The following data obtained in Table 2.

Table 2. The effect of adding *Spirulina sp.* powder to the feed

Variable	T1	T2	T3	T4	T5
Color Brightness (%)	1.04 ± 0.34 ^a	1.25 ± 0.26 ^{ab}	2.00 ± 0.16 ^{bc}	2.40 ± 0.04 ^c	2.68 ± 0.58 ^c
Survival Rate (%)	100	100	100	100	100

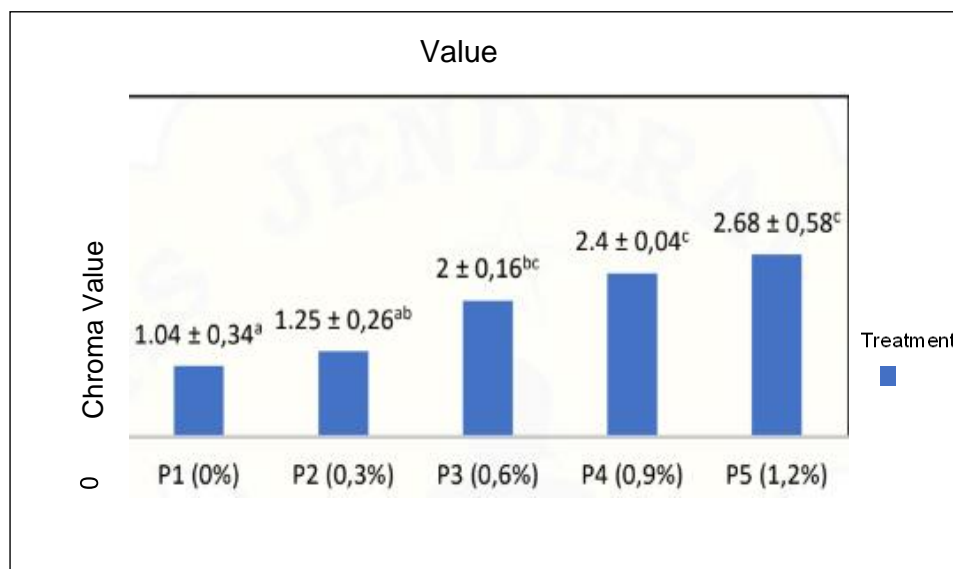


Figure 1. Chroma value of guppies

Fish Survival Rate (SR)

The survival rate during the study for all treatments had a percentage value of 100%. As a result, adding *Spirulina* pigment to the feed did not affect the fish's survival, the water quality parameters were always optimum during maintenance, and siphoning was done every day to ensure that the fish can adjust to the quality of the water and adequate feed. Following with the statement of Faturrahman *et al.*, (2020), that feed with a good shape and color can stimulate appetite in fish.

Discussion

Based on the results of water quality during the study, the temperature ranged from 25.8 to 26 0C, and the pH of the water ranged from 7.9 to 8.0 (Table 2).

Temperature can affect the metabolic processes of aquatic

organisms. Temperature is very influential on the life and growth of this aquatic biota. Water is a medium for fish to live, water quality plays a very important role in increasing the brightness of the fish's color. The water temperature obtained during the study was still considered normal for the growth and survival of guppies.

According to Pratama *et al.*, (2018) the ideal temperature for rearing tropical ornamental fish ranges from 25 – 32 °C. Temperatures that are too low can cause fish to be attacked by fungus, while temperatures that are too high can cause fish to stress and experience growth disorders. The pH value greatly determines the chemical processes in water, a pH that is too acidic or alkaline can

cause the color of the fish to turn pale and the fish's movement to slow down. During the research period, pH value was still relatively normal because the optimal pH range for tropical ornamental fish ranged from 6 to 9 (Boyd & Claude, 1990).

The results in Figure 1 indicate that each treatment increased color brightness, both with and without the addition of *Spirulina* powder. *Spirulina* powder contains carotenoids that can increase the number of chromatophores cells which causes guppies to have a brighter color. According to Sartikawati *et al.*, (2020), the increase in color brightness is different in each treatment because fish have different absorption rates for the type of color pigment and the dose given. The highest increase in color brightness value was found in treatment 4 (0.9%) and treatment 5 (1.2%). This shows that the higher the mixing of *Spirulina sp.* in the feed, the higher the brightness level of the fish color. The high value of color brightness in treatment 4 (0.9%) and treatment 5 (1.2%) influenced because guppy fish were able to utilize feed well. Giving adequate doses of carotenoids can increase the brightness of the fish's color so that the body provides better color expression. According to Sartikawati *et al.*, (2020), giving carotene

with different doses will affect the hormone system. Hormones have a limit to their ability to work According to Samara *et al.*, (2021), *Spirulina* has a good nutritional composition, namely protein, fat, and carbohydrates, which helps fish grow well and gain a brighter color.

According to Hadijah *et al.*, (2020), the carotenoid content in *Spirulina* can increase the number of chromatophores, resulting in more vibrant colors in ornamental fish (Fakhri *et al.*, 2020). *Spirulina sp.* is a source of natural carotenoids added in the feed that can be absorbed into the body of guppy fish, so it can increase the brightness of the color of guppy fish. According to Malinde *et al.*, (2018), adding a source of increasing color brightness in the feed will encourage an increase in color pigment in the fish's body, or at least be able to maintain color pigment in its body during the maintenance period. However, the results of different color observations in each treatment caused because fish have different absorption rates for the number of carotenoid sources given. The increase in color brightness influenced by chromatophoric cells located in the epidermis layer (Wallin, 2002). This happens because of changes in the chromatophores cells, namely

morphological and physiological changes. Morphological changes affect the addition and decrease in the number of chromatophores. Satyani & Sugito (1997) explained that this condition was influenced by the amount and composition of feed containing carotenoid sources in the feed, while physiological changes were caused by the movement of the chromatophoric pigment cells. The chromatophoric pigment cells that are scattered in the epidermis layer cause the pigment cell granules to absorb light perfectly, increasing the brightness of the color of the fish body to become brighter and clearer. The opposite happens when the pigment cells that gather near the epidermal nucleus can cause a decrease in the brightness of the fish's body color so that the fish's body color looks darker and fades (Kusuma *et al.*, 2012).

Spirulina sp. contains a common blue pigment called phycocyanin which can boost immunity (Nege *et al.*, 2020). Rosid *et al.*, (2019) explains that phycocyanin is a blue pigment that is structurally similar to beta-carotene, which is known to increase the action of the immune system and play an active role in protecting the body from disease. Carotene also naturally functions as a basic ingredient of vitamin A, supports thermoregulation or the process of

regulating body temperature, helps the formation of egg yolks in the reproductive process, and affects fish health.

Conclusion

Based on research, it can be concluded that the addition of *Spirulina sp* powder. The feed affects the brightness of the color of the guppy fish. Apart from that, the addition of *Spirulina sp* powder. the feed provides the highest chroma values, namely 0.9% and 1.2%.

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