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The Effect of Additional Dried Tubifex sp. in Commercial Feed Against Color Intensity of Guppy (*Poecilia reticulata*)

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Abstract

Guppy fish (*Poecilia reticulata*) is an ornamental fish in great demand because of its small size and beautiful color. The color of ornamental fish will generally fade due to a lack of carotenoids in their feed. This study aimed to determine the effect of giving *Tubifex* sp. in commercial feed to increase the color intensity of guppy fish and the best dose of *Tubifex* sp. The test fish used were male guppy fish strain *HB Red*. This study used a completely randomized design (CRD) method with five treatments and three replications, that is, commercial feed with the addition of *Tubifex* sp. with doses of 0% (P1), 2% (P2), 4% (P3), 6% (P4), and 8% (P5). The parameters observed were color intensity (chroma value), survival, and water quality. The addition of *Tubifex* sp. in commercial feed gave the effect with the highest yield on P5 at a dose of 8%, with an increase in color intensity (chroma value) of $4,21 \pm 0,25^{d}$. At P1, it gave an increase of $1,19 \pm 0,02^{a}$, P2 was $1,34 \pm 0,04^{a}$, P3 was $1,81 \pm 0,21^{b}$, and P4 was $2,88 \pm 0,18^{c}$. So, the best treatment is P5 (8%). Survival showed the results were not significantly different, that is, 100%. Water quality is included in the tolerance limit of fish with the results of temperature 26,6 – 27,4°C, pH 7,4 – 7,9, and DO 6,2 – 7,7 mg/L.

INTRODUCTION

Guppy (*Poecilia reticulata*) is a type of freshwater ornamental fish that is in great demand. The appeal of guppy fish lies in their small size and beautiful coloration. In addition, guppy fish have high adaptability so they are easy to rearing (Hasyim *et al*., 2018). Guppy fish have varied colors and patterns, especially male guppy fish which have brighter colors and wider caudal-fin than females (Lubis, 2014). A male guppy fish is in greater demand due to its color and wider caudal fin, they have a high economic value and are exported internationally (Hasyim *et al*., 2018).

According to Lubis (2014), the national market distribution of guppy fish in Indonesia is abundant in various regions, such as Java and Bali. In Indonesia, the *HB*

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red strain of guppy fish is a strain that is widely cultivated among farmers. This strain has a characteristic red-orange caudal fin with a half-black and half-silvery gray body. The color of ornamental fish is one of the most influential factors in the selling price of fish because color indicates the quality of ornamental fish (Khairunnisa *et al.*, 2020).

The color of ornamental fish will generally fade during rearing. Some of the causes of fish color fading are due to environmental stress, sunlight, water quality, and pigment content in feed (Khairunnisa *et al*., 2020). This is followed by Said *et al*. (2005), that the pigment content in the feed can cause the color changes that often occur in fish. Color in ornamental fish is caused by pigment cells, namely, chromatophores cells found in the dermis, on the outside, or under the scales (Maolana *et al*., 2017). According to Wibisono *et al*. (2018), the quality of chromatophores can be influenced by carotenoid content. However, aquatic animals cannot synthesize carotenoids in their bodies, so they must obtain these pigments from their food (Amin *et al.*, 2012).

The natural feed commonly used for ornamental fish is *Tubifex* sp. (aquatic oligochaete worm) because it has a high protein content and carotenoid pigments that can increase color intensity in ornamental fish and shrimp (Pursetyo *et al*., 2011). In nature, there are several types of carotenoids, but the most effective and dominant for coloring in fish are carotenoids from the xanthophyll class of astaxanthin types (Andriani *et al*., 2018). Mandal *et al*. (2010) stated in their research that the carotenoid pigment contained in *Tubifex* sp. is astaxanthin. Astaxanthin was 8000 ppm in live *Tubifex* sp. and 700 ppm in dry *Tubifex* sp.

The use of *Tubifex* sp. as feed is known to increase color intensity in dwarf gourami *Colisa lalia* (Saha and Patra, 2013), blue streak hap *Labidochromis caeruleus* (Wibisono *et al*., 2018), red cherry shrimp (Dermawan, 2019), and guppy fish (Mandal *et al*., 2010). Based on Dermawan (2019), feeding using *Tubifex* sp. as much as 7% resulted in the highest red color intensity in cherry shrimp among the other doses. The purpose of this study was to determine the effect of the addition of *Tubifex* sp. in commercial feed on increasing the color intensity of guppy fish and to determine the best dose of *Tubifex* sp. in commercial feed on increasing the color intensity of guppy fish.

METHODOLOGY

Ethical Approval

This research does not use ethical approval.

Place and Time

This research was conducted from April - May 2021 at the Laboratory of Fisheries and Marine Sciences Faculty, Jenderal Soedirman University.

Research Materials

The 15 guppies strain HB red used as test fish were 2.5 ± 0.5 cm in total length and 0.25 ± 0.05 g, which were obtained from ornamental fish cultivators in the Purbalingga area. The material used to attach the tubifex worms to the feed is a pellet binder. The equipment used in this research included an Aerator (LP 100), a digital scale with a precision of 0.1 gram, a pH meter (Hanna instruments), and a Dissolved Oxygen meter (Lutron DO-5510). The tool used to observe color changes in fish is a colorimeter application operated using a DSLR camera (Canon EOS 1500D) and a mini studio photo box used to take photos of research objects.

Research Design

This study used an experimental method with a Completely Randomized Design (CRD). The treatment was the addition of dry *Tubifex* sp. in artificial feed with different doses, namely T1 (0%), T2 (2%), T3 (4%), T4 (6%), and T5 (8%) with three repetitions. The guppies used for each treatment and repetition are three guppies (Pamulu *et al*., 2017).

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Work Procedure

Rearing Container Preparation

The guppies are kept in a container in an aquarium of 20 cm × 10 cm × 15 cm. The number of rearing aquariums is five units for each treatment. Each aquarium was washed and cleaned before it could be used. Then, the containers were arranged according to the design. Freshwater was filled in 2 liters, and aeration was installed in each aquarium.

Feed Preparation

The feed used during the study was commercial feed for ornamental fish (Topka, Taiwan) and dry *Tubifex* sp., which was purchased at an ornamental fish shop in Purwokerto, Banyumas. The dried *Tubifex* sp. was mashed first using a blender. At a dose of 2%, 2 grams of dried *Tubifex* sp. were used in 98 grams of commercial feed. At a dose of 4%, 4 grams of dried *Tubifex* sp. were in 96 grams of commercial feed. At a dose of 6%, 6 grams of dried *Tubifex* sp. were used in 94 grams of commercial feed, and at a dose of 8%, 8 grams of dried *Tubifex* sp. dried in 92 grams of commercial feed. Then *Tubifex* sp., based on the treatment, was mixed with pellet binder in a container until evenly mixed. Pellet binder is useful as an adhesive for fish feed that does not damage multivitamins and other supplements that will be combined with feed. The dose of pellet binder used was 3g/kg of feed (Yonarta *et al*., 2022). After that, add as much as 150 ml/kg of feed and stir. Then, commercial feed is placed in each container and remixed until it is sticky and evenly distributed. Feed that has been mixed with dry *Tubifex* sp., then air-dried at room temperature until dry.

Fish Rearing

The fish used for previous research will be acclimatized first. Fish were stocked in aquariums at a density of 3 individuals per aquarium. Guppy fish were kept for 30 days. The fish was fed twice daily at 8.00 and 16.00 WIB, as much as 3% of their biomass. The siphoning process was conducted every day at 17.00, which aimed to remove leftover feed and feces in the aquarium, which can cause poor water quality.

Color Intensity

Color intensity data collection from guppy fish was conducted once every seven days to determine the value of color intensity in guppy fish using the Colorimeter application. The results displayed were RGB (Red, Green, Blue), L* (lightness), a* (redness), b* (yellowness), Hue, and Chroma values. The value taken to determine the increase in color intensity in this study was the chroma value, referring to the method of Sukarman *et al*. (2017).

Survival Rate

Fish survival is the ratio of the number of fish that live at the end of the study to the number of fish stocked at the beginning of the study. The formula for calculating survival or survival rate, according to Dermawan (2019), is as follows:

$$SR = \frac{Nt}{No} x100\%$$

Where:

SR = Survival rate (%)
Nt = Number of living fish at the end of the study (fish)
No = Number of live fish at the start of the study (fish)

Water Quality

The water quality data observed during the study were temperature, pH, and dissolved oxygen, measured daily. Temperature and dissolved oxygen were measured using a DO meter (Lutron DO-5510, Taiwan), and pH measurements in this study were done using a pH meter.

Data Analysis

The color intensity and survival data were processed using the F test (ANOVA) to determine whether *Tubifex* sp. affected commercial feed for guppy fish. If there is a difference between treatments, proceed with the Least Significant Difference (LSD) Test at a 95% confidence level. Water quality data in

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temperature, pH, and dissolved oxygen were analyzed descriptively.

RESULTS AND DISCUSSIONS

Color Intensity

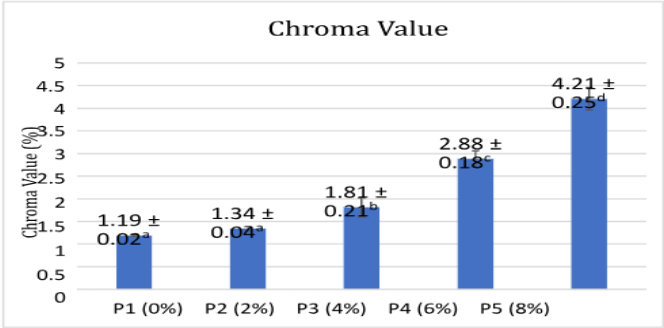

Figure 1. Guppy Fish Chroma Value (*Poecilia reticulata*).
Note: The difference in letter notation indicates a significant difference ($p<0.05$).

After the guppy fish rearing period for 30 days, it can be seen that the statistical analytical results (ANOVA) presented in Figure 1 showed an increase in the color intensity value (chroma value), which was significantly different between treatments. The color intensity (chroma value) increased in the feed treatment without adding *Tubifex* sp. In feed with the addition of *Tubifex* sp., each treatment had a different increase. P1 did not show a significant difference from P2, but significantly different from P3, P4, and P5. P3 shows a significant difference from P4 and P5. In P4, the results are significantly different from P5.

According to Mandal *et al*. (2010), the treatment of feeding *Tubifex* sp. live and dry conditions has the effect of increasing the carotenoid content in the body of guppy fish. Carotenoids are important in forming muscle pigmentation and color in ornamental fish (Swain *et al*., 2020). Feed is one of the factors that can determine color quality, especially feed containing pigments that can be used to increase color intensity for aquatic organisms that consume it (Dermawan, 2019). Research has proven that there is a difference in the color of the caudal fin of guppies in early rearing guppy and late rearing, which is a lighter red color. However, the brightness in each treatment is different because the carotenoid content is also different in each feed given, with the result of guppies that look brightest at P5.

According to research by Pursetyo *et al*. (2011), *Tubifex* sp. contains carotenoid pigments that can increase the intensity of color in the body of ornamental fish and shrimp. The carotenoid content in *Tubifex* sp. is in the form of astaxanthin (Mandal *et al*., 2010). Astaxanthin contains ten times more than other active compounds that influence fish color changes (Saputro *et al*., 2022). According to Mandal *et al*. (2010), *Tubifex* sp. has a fairly high carotenoid pigment content, around 8000 ppm. Astaxanthin is a carotenoid pigment with a red color (Amin *et al*., 2012). The greater the astaxanthin content contained in *Tubifex* sp., the greater the possibility that the red color will appear on the guppy fish (Pratama *et al*., 2021). So, the addition of *Tubifex* sp. in the feed in this study affected the color intensity of the HB red strain of guppy fish (Figure 1).

The research showed that the higher the dose of addition of *Tubifex* sp. in the feed, the higher the increase in the value of color intensity in fish. The lowest increase in color intensity value was at P1, P2, P3, and P4, and the highest increase in color intensity value was at P5. P1 showed an increase in the color intensity value of 1.19 ± 0.02 and at P5 of 4.21 ± 0.25. The increase in chroma values at P1 and P5 is significant because the carotenoid content

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given is also different, which means that the color of the guppy is different.

The increase in color intensity in each treatment depends on the amount of color composition in the feed (Malide *et al*., 2018). The color material in this study was the carotenoid type astaxanthin, found in *Tubifex* sp. In P1, there was no additional *Tubifex* sp. in the feed as an additional color material that served to increase the color intensity, so there was only a slight increase in the color intensity of the fish. In contrast to P5, which received a source of color material in the form of astaxanthin, which was higher in *Tubifex* sp., the increase in color intensity in fish was higher. The level of absorption of red color in fish was influenced by the number of carotenoid pigments contained in the feed (Amin *et al*., 2012).

This is following Dermawan's research (2019), which showed that a dose of *Tubifex* sp. high levels (7%) causes color pigments, in the form of carotenoids, to be consumed more and more, so that the increase in color intensity in fish is high. Paradea and Prabowo (2022) explained that the carotenoids contained in the fish feed would be absorbed and used by fish to produce pigments that can increase the color of the fish's body. The addition of *Tubifex* sp flour as a source of carotenoids certainly has a real influence in encouraging an increase in color pigment in the fish's body or at least can maintain the color pigment that is already present in the body of guppy fish (Subamia *et al*., 2010).

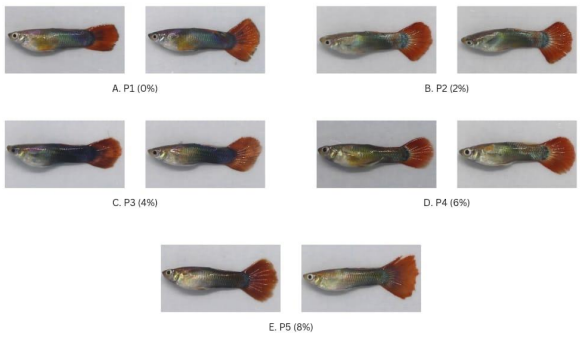

Figure 2. Early rearing guppy (left) and late rearing (right).

Survival Rate

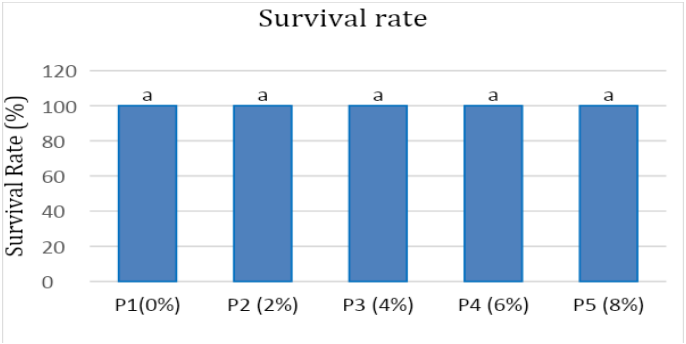

Figure 3. Guppy Fish Survival rate (*Poecilia reticulata*).
Note: The difference in letter notation indicates a significant difference ($p<0.05$).

The survival rate of guppy fish during the study showed that the results were not significantly different between each treatment, with a yield of 100%.

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These results explain that adding dry *Tubifex* sp. with different doses in the feed did not affect the survival of the fish. In addition to the influence of water quality that is always maintained and optimal stocking density of guppy fish so that there is no mortality in fish. According to research by Subandiyah *et al*. (2003), combining feed with *Tubifex* sp. with different doses did not show different fish survival results, namely with 100% survival results. In this research, the high protein content in commercial feed and dry *Tubifex* sp. causes fish survival during maintenance to produce a value of 100%. In commercial feed, the protein content is 46%, and in dry *Tubifex* sp., it is 57%.

Fish survival is caused by many factors, including fish stocking density, which affects survival, fish growth in motion competition, and oxygen consumption (Tarigan *et al*., 2014). In addition, during rearing, siphoning is carried out every day to reduce high ammonia levels caused by uneaten feed residue and fish waste so that the water is good and can support the survival of fish. Before maintenance, the fish were also adapted to environmental conditions and the feed provided. During maintenance, the condition of the fish is always maintained to stay healthy so that there is no mortality.

According to Subandiyah *et al*. (2003), the low stocking density reduces the competition for space for fish to get feed. Therefore, in this study, the density of fish in each aquarium is three guppies, where the density is not high. So that there is no competition of motion or competition in feed. In addition, quality control of water is carried out so the environment where fish live is always maintained.

Water Quality

Table 1. Water quality parameters.

Parameter	Range	Quality Standards
Temperature (°C)	26,6 – 27,4	26 – 30 *
pH	7,4 – 7,9	6 – 9**
DO (mg/L)	6,2 – 7,7	≥ 4 ***

Sources: *Panjaitan *et al.* (2016), **Boyd (1990), ***Susanto (1990).

Based on Table 1, the results of measuring water quality for 30 days of guppy fish rearing were temperatures ranging from 26.6 – 27.4°C, pH ranging from 7.4 – 7.9, and dissolved oxygen ranging from 6.2 – 7.7 mg/L. Temperature is one factor that can affect guppy fish's level of life (Matondang *et al*., 2018). The temperature in the water was around 26.6 – 27.4°C, including the tolerance range for raising guppy fish. Following the statement of Panjaitan *et al*. (2016), in general, guppy fish can live normally in a temperature range of 26 – 30°C. Temperatures that are too low can cause fish to be attacked by fungi, while temperatures that are too high can cause fish to be stressed and experience growth disorders (Pratama *et al*., 2018). Temperature also indirectly affects the solubility of oxygen in the water. The higher the water temperature, the lower the solubility of oxygen in the water, and vice versa. If the water temperature increases drastically, it will cause death (Priyono *et al.*, 2013).

The pH value during the study ranged from 7.4 to 7.9. This condition is still quite good because the optimal pH range for tropical ornamental fish ranges from 6 to 9 (Boyd, 1990). This also follows Utomo (2008), who states that a good pH value for guppy fish life is around 7.0. A pH value that is too acidic or alkaline can cause the color of the fish to turn pale and the fish's movement to slow down

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(Pratama *et al*., 2018). The pH value affects the toxicity of materials or other chemical factors, such as ammonia (Matondang *et al*., 2018). The pH value affects carbon dioxide and alkalinity. The higher the pH, the higher the alkalinity value and the lower the free carbon dioxide (Hasyim *et al.*, 2018).

Dissolved oxygen (DO) is a parameter that determines the dissolved oxygen content in the water in the maintenance container. Dissolved oxygen determines fish's survival rate and stress levels, which can affect fish mortality. Dissolved oxygen content during maintenance showed results between 6.2 – 7.7 mg/L. According to Susanto (1990), the minimum oxygen content for guppy fish is four mg/L; dissolved oxygen during rearing is included in the tolerance limit of guppy fish life. Aquatic organisms need oxygen in sufficient quantities so they are not stressed and are not susceptible to disease and parasites. In water conditions with extreme dissolved oxygen content, it can cause sudden and mass fish death (Matondang *et al*., 2018). If the dissolved oxygen level in the water is below two mg/L, it will cause the aquatic organisms that live in these waters to die (Panjaitan *et al.*, 2016).

CONCLUSION

The addition of dry *Tubifex* sp. in commercial feed affects the red color intensity of the guppy fish (*Poecilia reticulata*). The best dose for increasing the color intensity of guppies is 8%, with an increase of the chroma value of 4.21 ± 0.25.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this publication.

AUTHOR CONTRIBUTION

HIN: Methodology, K, Y, and M: Writing – original draft, preparation, RF and PHTS: Conceptualization, Project administration, Writing.

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