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EFFECT OF ASTAXANTHIN ON THE COLOR INTENSITY OF GUPPY (*Poecilia reticulata*)

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Abstract

Guppy (*Poecilia reticulata*) is one type of ornamental fish which is in great demand because of the beautiful variety of colors. Color of ornamental fish can be brighter when supplemented with pigments-rich feed. Astaxanthin is a liposoluble carotenoid and a reddish-orange pigment. The study aims to determine the effect of Astaxanthin supplement in feed on the color brightness of guppies and to find the best dose of Astaxanthin supplement in pellets on the color brightness of guppies. The method used in the study was completely randomized design (CRD) consisting of five treatments (Astaxanthin supplements of 0%, 2%, 3%, 4% and 5%) and three replications. HB Red strain of male guppy was used as experimental fish with a body length of 3±0.2 at a density of 3 fish per aquarium. Feeding was done twice a day and siphoning was done every afternoon. The parameters observed were chroma value, survival rate and water quality. The results show that the supplementation of Astaxanthin increase the brightness of guppies and the best dose of Astaxanthin supplement in pellets was 5% with an increase in chroma value of 4.82%. The survival rate obtained was 100%. Water quality parameters such as temperature are 26-27 ℃ and pH of 6-7 and dissolved oxygen (DO) was 6.95-7.93 ppm were maintained during the study.

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Introduction

Ornamental fish are the fish having their own characteristics. The attraction of ornamental fish can be determined based on their brilliant color, shape and physical completeness, behavior and health condition or stamina. Ornamental fish is used as a decoration for aquarium which is considered an art for the enthusiats (Vasantharajan, 2023). Guppy (*Poecilia reticulata*) is one of the freshwater ornamental fish commodities having high adaptability and is easy to cultivate. Guppies are small freshwater ornamental fish. The fish have various strains of about more than 300 species with different sizes, color patterns, and tail shapes on their bodies, especially in male fish. Meanwhile, female fish has monotonous color (García *et al.*, 2023; Sukrillah *et al.*, 2014).

One of the Guppy strains easily found

in Indonesia is the *HB Red* strain Guppy. This variety is easily found by the farmers of guppy ornamental fish because they are in great demand by the community as decorations in aquarium. HB Red strain Guppy is unique among the other guppy strains since it has quite attractive color pattern on its body and tail which is combined in black and blue on its body; and red and brown on its dorsal fins and tail, as well as a slight yellow around the body behind the gills. Brightness of guppy colors increase when the fish grows towards the reproductive phase; and the fish genetic condition is an internal factor affecting the color of guppies (Blattner et al., 2012). In addition to internal factors, the color of Guppy can also be affected by several external factors such as water quality, light intensity and feed containing carotene (Almaas & Harlita, 2023).

Feed is the main component in ornamental fish farming. The feed consumed may support growth and survival of the fish, therefore the feed must be in accordance with the fish needs both in quantity and quality. Kaur and Shah (2017) stated that there are various ways to improve color quality, one of which is the addition of color pigments through the fish feed. The pigments used can be either synthetic pigments or natural pigments. As stated by Basiony et al. (2022), aquatic animals cannot synthesize carotenoids in their bodies; therefore, this pigment can be generated by enriching the content of pigment cells in the fish's body through feeding containing Astaxanthin (Kiswara et al., 2020).

The feed consumed by fish is the main factor affecting the color of ornamental fish; therefore, it is necessary to give sufficient feed supporting the enhancement of the fish's color (Amaya & Nickell, 2015). The pigment-forming components are carotenoid, which is natural pigment component. Carotenoids can be generated from both chemicals and natural ingredients originated from plants or animals (Maoka, 2020). Fish color can be affected by carotene substances which cannot be synthesized inside the body of aquatic animals, hence external supplement is necessary, one of which is enriching the feed with carotene content (Nakano, & Wiegertjes, 2020). Astaxanthin is a source of carotene which provides an effective enhancement of red and orange colors (Jiang et al., 2019). The supplement of Astaxanthin to the pellet feed is supossed to increase the color intensity value of Guppy. The purpose of this study is to determine the effect Astaxanthin supplement in enriching pellet feed on the color brightness of Guppy

(*Poecilia reticulata*) and the best dose of Astaxanthin supplement in enriching pellet feed on the color intensity of Guppy (*Poecilia reticulata*).

Materials and methods

Preparatory stage was carried out to prepare Guppy rearing media and feed preparation by adding Astaxanthin according to the concentration specified in the treatment. The implementation stage consisted of fish rearing and the provision of each fish according to the treatment. The analysis phase was carried out by observing color intensity, survival rate and water quality.

In this study, 5 doses of Astaxanthin were added to 100 g of commercial feed i.e. 0%, 2%, 3%, 4%, 5% for 30 days of rearing to observe color changes effect of the test fish. The main variables observed were the intensity of the color and the supporting variables were the survival (survival).

Feed Production

Astaxanthin used in this study was in the form of dry flour. Each dose of Astaxanthin was added to the commercial feed of ornamental fish. Mixing stages of Astaxanthin in the feed are as follows: 1) Astaxanthin flour according to the dose was mixed with progol (2-3 g/kg feed) in a container and stirred evenly; 2) Water at a dose of 150 ml Kg^{-1} feed was added to the Astaxanthin flour that was mixed evenly with progol and then set down for 10 minutes; 3) Feed was poured into a container of Astaxanthin flour with progol which had been dissolved in water. Then, the mixture was stirred until the whole Astaxanthin flour was evenly sticky on the feed; 4) If the Astaxanthin flour was already sticky, the mixture was then air-dried for 30–60 minutes. If there is a change in color and odor during drying process, the feed should be disposed and remade.

Container Preparation

The pisciculture container used was 15 pieces of aquarium 25×10×20 cm length that had been washed and dried. The aquariums were filled with water; then aeration system was installed as an oxygen supplier. Before placed in the pisciculture containers, the fish were acclimatized in a holding tank for 3 days. The process of acclimatization aims to allow the fish to adapt to the new environment and the type of feed. This treatment was carried out for 3 days in a holding tank. After that, sorting was done to choose healthy fish. The stocking density used was 3 fish/aquarium (Hasyim *et al.*, 2018), the number of fish required was 45 fish in the volume of 4 liters water. The pisciculture was carried out for 30 days.

Water control system was carried out by siphoning and measuring water quality every day. Total volume of water siphoned was 10% in the pisciculture container. Water quality parameters were also carried out to determine the water condition. The measurement of water quality included temperature, pH and dissolved oxygen (DO). Measurement of water quality, temperature, pH and dissolved oxygen (DO) were done once every day.

The feed was provided twice daily at 8 a.m. and 4 p.m. (Local time) for each treatment. The amount of feed for Guppy was calculated as 3% of fish body weight. The average weight of Guppy was 0.28 g, thus the amount of feed provided per day was 0.05 g

Research Parameters

The parameters observed in this study were the value of color intensity (Chroma), fish survival and water quality (temperature, pH, and dissolved oxygen (DO).

Color Intensity (Chroma)

Measurement of the color intensity value of Guppy was done using Colorimeter application, the application works with the value or number of Light (light or dark), a*, b*, Chroma and Hue. Chroma value is calculated using the formula (1) of Nickell and Bromage (1998):

$$C^* = \sqrt{a^2 + b^2} \quad \ldots\ldots\ldots\ldots\ldots (1)$$

Description :

C* = Chroma Value (%)

a* = Reddish color

b* = Yellowish color

Survival Rate

Survival of Guppy is the ratio of the number of fish living at the end of the observation with the number of fish stocked at the beginning of pisciculture. According to Effendi (1997), the formula (2) used to measure survival is:

$$SR = \frac{Nt}{N0} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots (2)$$

Description :

SR = Survival Rate (%)

Nt = Number of live fish at the end of the study

N0 = Number of live fish at the beginning of stocking.

Water quality

Water temperature was measured every day at 8 a.m (Local time) using a digital thermometer. Digital thermometer was dipped in the water for 1 minute. When the scale showed a constant number, it was then noted down by the researchers. pH measurement was carried out every day at 8 a.m (Local time). pH meter was dipped in the aquarium until showing a constant number, it was then noted down by the researchers. Dissolved oxygen was measured using a DO meter. The sensor part was dipped in the

aquarium. When the scale showed a constant number, it was then noted down. Dissolved oxygen was measured daily until the end of pisciculture.

Data analysis

Data of the study results were processed using analysis of variance (ANOVA) combined with F test to determine whether or not there was an effect of giving Astaxanthin flour in artificial feeds on the color intensity of Guppy (*Poecilia reticulata*). If there were a difference of color intensity among treatments, it was then proceed with BNT test at 95% confidence level. The data of fish survival was processed using Arschin Transformation Test, while the water quality was analyzed descriptively.

Results

Based on the result of the study, the addition of Astaxanthin to feed pellets in increasing the value of color intensity of Guppy (*Poecilia reticulata*) has an effect on the brightness of fish color and water quality. The data are presented in Table 1.

Color Intensity (Chroma)

The data observed in this study was the amount of chroma value detecting the content of carotenoids in the feed as an indicator of the increase of fish color brightness. The higher chroma value indicates the brighter color of the fish (Sholichin *et al.*, 2012). In Figure 1, the results of statistical analysis (ANOVA) on the color intensity shows a significant difference (T<0.05). The P1 value was not significantly different from P2 and significantly different from P3, P4 and P5.

Table 1. Effect of Astaxanthin addition on chroma value in guppy fish

Variabel	**P1**	**P2**	**P3**	**P4**	**P5**
Color intensity	0,87±0,05[a]	1,35±0,11[a]	2,46±0,40[b]	3,88±0,02[c]	4,82±0,15[d]
Survival rate	100%	100%	100%	100%	100%
			Water Quality		
Temperature	26-27°C	26-27°C	26-27°C	26-27°C	26-27°C
pH	6-7	6-7	6-7	6-7	6-7
Dissolved Oxygen	6,95 ppm	6,95 ppm	6,95 ppm	6,95 ppm	6,95 ppm

Table 2. Increased color intensity value (Chroma).

Treatment	**week**				**Average**	**Total**
	1	**2**	**3**	**4**		
P1 (0%)	0,25	0,25	0,22	0,15	0,22	0,87
P2 (2%)	0,38	0,35	0,34	0,29	0,34	1,35
P3 (3%)	0,68	0,58	0,66	0,53	0,61	2,45
P4 (4%)	1,14	0,90	0,87	0,97	0,97	3,88
P5 (5%)	1,27	1,19	1,04	1,31	1,20	4,82

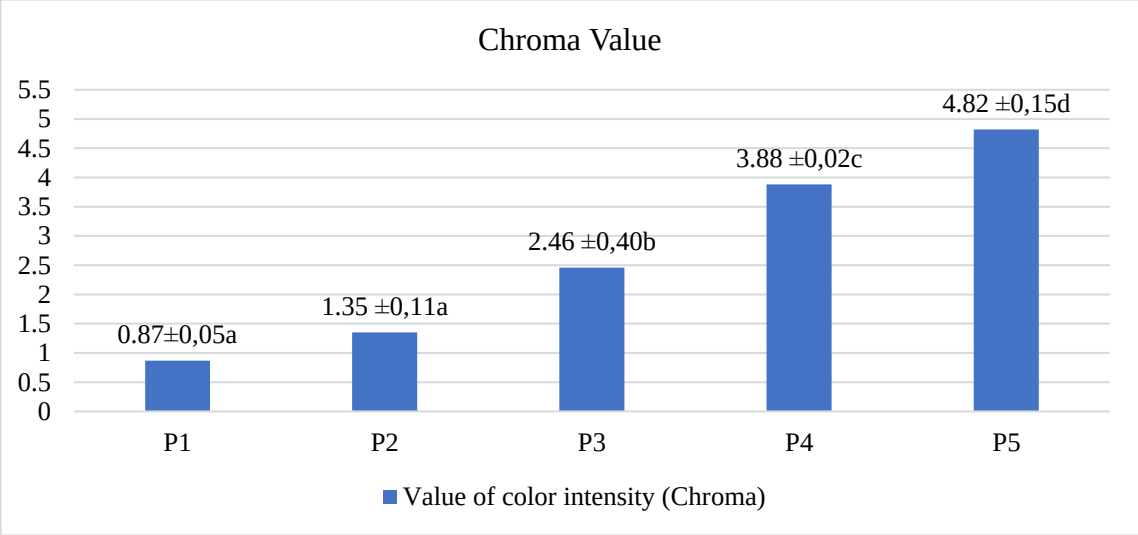

Figure 1. Value of color intensity (Chroma)

The best chroma value in the P5 treatment was around 4.82 ± 0.15. In guppy fish, Astaxanthin can increase the intensity of the chroma value. Hence, the addition of Astaxanthin as carotene content in feed enrichment has a significant effect.

The increase rate of color intensity value of Guppy observed in this study is presented in Table 2.

Survival rate

Survival rate of the fish is the ratio between the population of living organisms at the initial stocking and the population of living organisms at the end of observation stated in percentage. In the study of enriching carotenoid content in the fish feed by the addition of Astaxanthin, there was no mortality found in all treatments of Guppy. Data of survival rate is illustrated in Figure 2.

Water quality

Water is the medium in which aquatic organisms live. Water quality is one of the important factors to be considered because it is a determinant for the organisms life support. Fish rearing media must be maintained to ensure that the water quality

Table 3. Water quality

Parameter	**value**	**Optimum value**
Temperature	26-27°C	22-30°C (Apriyani *et al.*, 2019)
pH	6-7	5-8 (Nixon, 2004)
Dissolved Oxygen	6,95-7,93 ppm	≥4 - ≤9 ppm (Effendi, 2003)

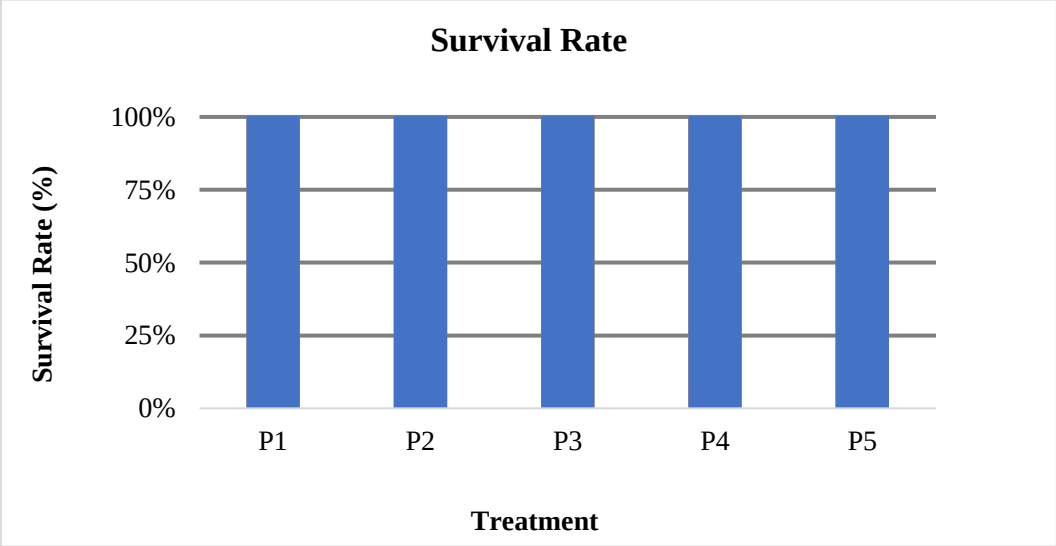

Figure 2. Survivals of fish

meets the quality standards; hence it will support the fish's ability to survive and grow well. Water quality parameters observed in this study were temperature, pH, and DO presented in Table 3.

Discussion

Based on results, treatment 1, which is the control treatment, has an increase of 0.87 ± 0.05^{a}, the increase of color intensity value in treatment 1 is considered low due to the lack of carotenoid levels in the feed consumed by fish causing the fish body does not get optimal carotene substances to increase the brightness of the fish body color. Sources of carotenoid for guppies can be obtained from both natural feeds and additives mixed into artificial feeds. Natural food is phytoplankton produced from the photosynthesis process of benthic. Artificial feed (pellets) does not contain carotenoid as a source of fish color brightness, however, it can be mixed with additional ingredients i.e. Astaxanthin (Destiyanti et al., 2023; Lalita et al., 2022).

The highest increase in color intensity value was found in treatment P5 which the pellet feed was enriched with 5% Astaxanthin (4.82±0.15^{d}). The dose given was based on the need for carotene level by male guppy to increase the color brightness; it was due to the addition of carotene substances (Astaxanthin) into the pellet feed can be optimized by fish to increase the color brightness. According to Rahmawati *et al.* (2016), the increase of fish color intensity depends on the amount of feed, especially composition of the feed, the more carotenoid added to the feed, the higher increase of color

intensity value resulting in brighter color of the fish body. The increase in color intensity occurred due to the addition of carotenoid sources (Astaxanthin) to the feed in the right dose to meet the needs of Guppy.

The increase rate of color intensity value of Guppy observed in this study is presented in Table 2. Treatment P1, a control treatment, has an average increase of 0.22% in each measurement, an increase of color intensity value in treatment P1. The highest increase of color intensity value was found in treatment P5 which was adding pellet feed with 5% Astaxanthin, which was 1.20% in each measurement. In general, Guppy will directly absorb the carotenoid sources contained in the feed and use it as a pigment to increase their bodies' color intensity. Excessive addition of Astaxanthin will not result in optimal color of Guppy, thus an effective dose is needed to increase color intensity of the fish. It is in accordance with the statement of Meiyana and Minjoyo (2011) that the dose of Astaxanthin powder must be noticed, because excessive doses of Astaxanthin will result in the immune system decrease of the fish and deficient color of the fish's body.

Survivals value during the study in both control and treatment was 100%. It shows that the addition of synthetic pigment Astaxanthin to the pellet feed did not affect the survival of the test animals; moreover, water parameters were always optimum and stable during the study, as well as, stocking density was optimal for fish movement space. This is in accordance with the statement of Rahmawati *et al.* (2016) that the addition of carotenoid sources, such as Astaxanthin, did not have certain effects on the fish survival. There are several factors affecting the fish survival, including cannibalism, predation, stress, lack of feed and age. Low mortality affects the fish survivals. High mortality rate during the study indicates low survivals, and vice versa (Rudiyanti & Dana, 2009). Mortality may occur naturally in organisms with insignificant growth rate (Rapi et al., 2021). Parameters of water quality and density of organisms in a scope are also important factors in determining the survival of fish. Good quality is in accordance with the biological requirements of fish or it is within tolerance for fish to live. As stated by Effendi (2003), there are many influencing factors to the survival of fish in waters; including density and water quality. Thus the possibility of the fish mortality is insignificant.

Based on water quality results, there are several supporting factors of water quality ensuring the survival of fish and optimize the

results of pisciculture, namely water quality comprising temperature, pH, salinity, ammonia and DO. Water quality is considered important as it may affect the optimization of growth, reproduction, and the fish health (Akhter et al., 2021). In addition to water quality, there are other factors affecting the fish color. Subamia et al. (2010) stated that when fish are stressed or shocked, granules in the epidermis will gather in the middle of the cells causing the fish color to be disappear or fade.

In the effort to increase color intensity value of the fish through the addition of carotene into the feed, temperature is one of the external factors must be considered during the study since temperature will affect the metabolic rate of fish. Extreme temperature changes in the water will cause energy changes for growth and oxygen consumption levels resulting in the fish mortality. Temperature recorded during this study ranged from 26-28°C. This temperature leads to the optimal and stable growth of the fish to maintain the stability of growth performance in the fish body. It is in line with the statement of Apriyani *et al.* (2019), the optimal water temperature for Guppy ranges from 25-28°C. In this temperature range, Guppy have a good appetite and the tail growth will be maximal. Furthermore, it is supported by the statement of Diana *et al.* (2017), optimal temperature for the growth of tropical ornamental fish ranges from 25 - 32°C.

The next water quality parameter is pH. According to Arifin et al. (2018), the high and low pH value in water is influenced by the amount of waste in the water, especially from feed residues and fish metabolism residues. pH value will significantly determine the chemical process in water, since pH is an indicator to determine the nature of water. When the pH is too acidic or alkaline, the color of Guppy will be diminished to and causing slower fish movement (Sari *et al.*, 2012). pH value recorded during the pisciculture ranges from 6 - 7. The pH value is believed to be optimum for the life of Guppy and their growth. As stated by Nixon (2004), guppies will survive in the pH range of 5 – 8. It is based on Effendi's opinion (2003) that pH quality standard value in waters generally ranges from 6-7. It is necessary to maintain the pH conditions in the waters to remain stable because if the water conditions have a low or acidic pH value, the water will be toxic and it is harmful for the live of organisms in the water. Manik and Eddy (2003) stated that heavily polluted waters have pH value of less than 5 or more than 9.

Dissolved oxygen is an indicator of the content of dissolved oxygen in the water. Aquatic organisms truly need oxygen in sufficient quantities to create optimum conditions for fish and avoid stress, tissue hypoxia, anorexia, unconsciousness, susceptible to disease and parasites. Dissolved oxygen in water is needed by living organisms to carry out respiration or breathing. One of the consequences of the lack of dissolved oxygen levels in the waters will cause a decrease in fish appetite and lead to disease deployment. In ornamental fish, especially guppies, the lack of oxygen will cause the limp guppies, appetite loss and the tail will fade. Oxygen levels below 5 ppm can cause difficulty in breathing and appetite loss of Guppy. To obtain optimal growth, the oxygen content must be maintained at least above 5 ppm. If the oxygen content remains at 3 or 4 ppm for a long time, fish will stop eating and growing (Ramadhani *et al.*, 2020). Dissolved oxygen value recorded during rearing ranged from 6.95 to 7.93 ppm, so as to create optimal growth conditions for guppies. As stated by Susanto (1990) that dissolved oxygen content for Guppy was at least 4 ppm. Dissolved oxygen content during the study was classified as good for the growth and survival of Guppy.

Conclusion

Based of this study, it can be concluded that the addition of Astaxanthin in the pellet feed enrichment had significant effect on the color brightness of Guppy (*Poecilia reticulata*). It was indicated by the increase of color intensity value of the fish resulted in an increase of the fish color brightness. The best dose of Astaxanthin addition in the pellet feed enrichment is 5% with an increase of chroma value of 4.82% and 100% survival rate; while the water quality as follows, temperature of 26 – 27°C, pH of 6 – 7 and dissolved oxygen of 6.95 ppm.

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