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The Effect of Astaxanthin Addition in Commercial Feed on Color BrightnessLevel and Growth of Koi Fish (*Cyprinus carpio*)

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ABSTRAK

Ikan koi (*Cyprinus carpio*) merupakan salah satu ikan hias yang banyak diminati karena keindahan bentuk badan dan warnanya yang beranekaragam, memiliki prospek usaha yang bagus, dan permintaan yang terus meningkat dari tahun ke tahun dengan produksi yang dihasilkan mencapai 36% pada tahun 2015-2019. Pola dan kecerahan warna kulit yang cemerlang dari koi merupakan alasan ikan hias ini diminati. Upaya yang dapat dilakukan untuk mempertahankan dan meningkatkan kualitas kecerahan warna pada koi yaitu dengan cara memberikan pakan yang mengandung sumber pigmen warna. Salah satu sumber pigmen warna yaitu astaxanthin Astaxanthin merupakan salah satu senyawa dari kelompok pigmen karotenoid yang dapat digunakan sebagai suplemen pakan untuk meningkatkan warna ikan. Tidak hanya untuk meningkatkan kecerahan warna pada ikan, penambahan astaxanthin juga memberi pengaruh terhadap pertumbuhan.

Tujuan penelitian ini adalah untuk menganalisis pengaruh penambahan astaxanthin dalam pakan komersil terhadap tingkat kecerahan wana dan pertumbuhan ikan koi (*C. carpio*). Penelitian ini menggunakan metode eksperimen, rancangan acak lengkap (RAL) yang terdiri atas 5 perlakuan dengan 3 kali ulangan. Perlakuan yang digunakan adalah perlakuan masing-masing dengan penggunaandosis astaxanthin 0, 50, 100, 150, dan 200 mg/kg pakan. Pengukuran kecerahan warna menggunakan *Toca Color Finder* (TCF) yang kemudian diberi skor. Ikan uji yang digunakan adalah ikan koi jenis karasi yang memiliki warna dasar tubuhnya kuning orange dengan bobot rata-rata 10-14 g/ekor. Ikan koi dipelihara dalam toples plastik berukuran 5 l dengan padat penebaran 3 ekor/l. Pemeliharaan dilakukan selama 30 hari dengan metode pemberian pakan *at satiation*.

Hasil penelitian menunjukkan bahwa penambahan astaxanthin dalam pakan komersil memberikanpengaruh nyata (P<0,05) terhadap tingkat kecerahan warna, total konsumsi pakan (TKP), konversi rasio pakan (FCR), efisiensi pemanfaatan pakan (EPP), protein efisiensi rasio (PER), dan laju pertumbuhan relative (RGR), namun tidak berpengaruh (P>0,05) terhadap nilai kelulushidupan (SR) ikan koi. Berdasarkan hasil tersebut didapatkan dosis terbaik penambahan astaxanthin yaitu pada dosis 200 mg/kg (Perlakuan 5) yang menghasilkan nilai rata-rata kecerahan sebesar 15,83±0,76, FCR sebesar 2,76±1,00 g, EPP sebesar 30,24±5,37%, PER sebesar 35,73±3,27%, dan nilai RGR sebesar 0,68±0,19% per hari.

***Kata kunci*:** ikan koi, astaxanthin, pakan komersil, kecerahan warna, pertumbuhan

ABSTRACT

Koi fish (Cyprinus carpio) are one of the most popular ornamental fish because of their beautiful body shapes and diverse colors. They offer promising business prospects, and the demand for them continues to increase each year, with production reaching 36% between 2015 and 2019. The striking patterns and brightness of koi skin are among the reasons why this ornamental fish is so sought after. Efforts to maintain and enhance the brightness of koi coloration can be made by providing feed that contains pigment sources. One such pigment source is astaxanthin. Astaxanthin is a compound from the carotenoid pigment group that can be used as a feed supplement

to enhance fish coloration. In addition to enhancing color brightness in fish, the addition of astaxanthin also influences growth.

The aim of this research is to analyze the effect of adding astaxanthin to commercial feed on the color brightness level and growth of koi fish. This study uses an experimental method with a completely randomized design (CRD) consisting of 5 treatments with 3 replications each. The treatments involved different doses of astaxanthin: 0, 50, 100, 150, and 200 mg/kg of feed. Color brightness was measured using the Toca Color Finder (TCF) and then scored. The test fish used were koi of the karasi type, with a basic body color of orange-yellow and an average weight of 10–14 g each. The koi were kept in 5-liter plastic jars with a density of 3 fish / l. The maintenance period lasted for 30 days, with feed given at satiation.

The results showed that the addition of astaxanthin to commercial feed had a significant effect ($P<0.05$) on color brightness level, total feed consumption (TFC), feed conversion ratio (FCR), feed utilization efficiency (FUE), protein efficiency ratio (PER), and relative growth rate (RGR), but had no significant effect ($P>0.05$) on the survival rate (SR) of koi fish. Based on these results, the best dose for astaxanthin supplementation was 200 mg/kg (Treatment 5), which resulted in an average brightness score of 15.83±0.76, FCR of 2.76±1.00 g, FUE of 30.24±5.37%, PER of 35.73±3.27%, and an RGR value of 0.68±0.19% per day.

Keywords: *koi fish, astaxanthin, commercial feed, color brightness, growth*

INTRODUCTION

Koi (*Cyprinus carpio*) is one of the most popular ornamental fish due to the beauty of its body shape and diverse colors, and it is also believed to bring good luck to koi enthusiasts in Indonesia (Lesmana, 2007; Iskandar *et al*., 2021). According to Purnomo (2015), koi has become one of the most sought-after ornamental fish in Indonesia in recent years. In addition to being a favorite, koi also has promising business prospects, with steadily increasing demand year after year. According to DJB data (2016), ornamental fish production in 2016 reached 1.9 billion fish, while the target for 2017 was 2.1 billion fish, with koi accounting for the highest proportion of Indonesian ornamental fish from 2015 to 2019, contributing up to 36% of total production. The patterns and brilliant color brightness of koi skin are reasons why this ornamental fish is favored by the Indonesian public. Its color characteristics are distinguished by several color patterns, color compositions, and markings on different parts of the koi's body (Hasan, 2021). The colors found in koi are diverse, ranging from red, white, yellow, black, or their combinations, creating the fish's aesthetic value. The continuously increasing demand each year is one of the advantages for koi ornamental fish farmers. Color brightness becomes the main indicator of a koi's beauty, making it necessary for breeders to enhance its color quality. The more beautiful the skin color, body shape, and the livelier the fish's movements, the higher the selling price of the koi. The beautiful colors in fish occur due to the number and placement of pigment cells (chromatophores) in the epidermal layer (Sari *et al*., 2012).

Efforts that can be made to maintain and improve color brightness quality in koi include providing feed containing pigment sources. One such pigment source is carotenoids. Carotenoids are pigments that produce yellow, orange, and red colors (Maleta *et al*., 2018). Naturally, carotene functions to provide or sharpen the appearance of color in ornamental fish (Melati *et al.*, 2017). Efforts to add carotenoid sources to feed can be done by giving feed that contains astaxanthin (carophyll pink) (Sitorus, 2014; Fikrillah *et al.*, 2021). Astaxanthin is one of the compounds from the carotenoid pigment group that can be used as a feed supplement to enhance fish coloration (Sofian *et al*., 2019). Carotenes are divided into two groups: nutritionally active carotenes such as alpha, beta, and gamma carotene, and non-nutritionally active ones such as astaxanthin and kataxanthin (Subamia *et al*., 2010; Fikrillah *et al.,* 2021). Various studies on using astaxanthin to enhance fish color brightness through feed combinations have been widely conducted. According to Fikrillah *et al.* (2021), maintaining koi with feed supplemented with astaxanthin powder at different doses has a significant effect on koi color brightness, with a color brightness index ranging from 5-7. Adding color-enhancing sources to feed will stimulate an increase in pigment in the fish's body, or at least help maintain existing pigments during the rearing period (Wayan *et al.*, 2010; Diansyah *et al*., 2019). In addition to improving fish color brightness, the addition of astaxanthin also influences growth. This is supported by Yulianti *et al.* (2014), who stated that increased fish growth may be influenced by the protein content in astaxanthin at appropriate doses, so feed supplemented with astaxanthin can affect growth rates.

MATERIALS AND METHODS

1. Tools and Materials

The equipment used in this research includes 15 plastic jars with a capacity of 5 l as containers for keeping the koi, a digital scale for weighing the test fish, and 5 jars of approximately 800 ml each, labeled according to treatment codes, for storing feed. There are also 5 basins used to mix feed according to each treatment, a scoop net for catching the test fish to be observed, and an aerator to supply oxygen. A thermometer, pH meter, and DO meter are used to measure water quality. A siphon hose is used for siphoning, a digital camera is used for documentation, and writing tools are used for recording research data. Meanwhile, to determine color enhancement, a TCF (Toca

Color Finder) color measurement device is used. The materials used in this research are koi (*Cyprinus carpio*) sourced from BBI Balekambang, measuring 8-10 cm in length and male, totaling 45 fish. Clean water is used as the living medium for the test fish. Astaxanthin (Carophyll DSM 10%) about ±500 mg is used as the coloring agent. The commercial feed used is Ms Prima Feed fish pellets, which are commonly used at BBI, totaling about ±5 kg, and progol is used as an adhesive for the coloring agent on the feed.

2. Preparation of Test Feed

The feed used during the study was commercial feed, which was then mixed with astaxanthin according to the treatment dosage. The method for mixing astaxanthin into the feed is supported by the research of Putra *et al.* (2020), where the astaxanthin powder for each treatment dose is first mixed with a binder, namely progol (2-3 g/kg of feed) in separate containers and stirred until evenly distributed. This mixture is then combined with water at 150 ml/kg of feed, and stirred until homogeneous. The process of mixing astaxanthin is shown in Figure 1. Next, pour and mix the mixture evenly into 1 kg of pellets according to the treatment dosage. After that, the feed is dried for 30-60 minutes.

Figure 1. Mixing Astaxanthin and Progol Before Being Combined into Commercial Feed According to the Treatment Dosage.

3. Maintenance of Test Fish

The fish were placed in jars with a stocking density of 3 fish per jar. Before the treatment was applied, the fish were first fasted for 24 hours in order to eliminate any remaining feed in their bodies. Next, the fish were weighed to obtain initial weight data, and their color brightness was observed as a comparison for the end of the study before being placed into the containers. Maintenance and observation of the test fish were conducted for 30 days, with feeding given at satiation, three times a day at 08:00, 12:00, and 16:00 (Amalia *et al.*, 2013).

4. Research Method

The experimental design used in this study is a Completely Randomized Design (CRD) with 5 treatments and 3 replications, based on the research of Putra *et al.* (2020), with the following doses:

Treatment A : The treatment of adding astaxanthin 0 mg/kg of commercial feed
Treatment B : The treatment of adding astaxanthin 50 mg/kg of commercial feed
Treatment C : The treatment of adding astaxanthin 100 mg/kg of commercial feed
Treatment D : The treatment of adding astaxanthin 150 mg/kg of commercial feed
Treatment E : The treatment of adding astaxanthin 200 mg/kg of commercial feed

According to Lien *et al.* (2018), the coloration of ornamental fish depends on the source of pigment, whether it is natural or synthetic, with an appropriate dosage of 100-250 ppm.

5. Data Collection

5.1. Observation of Color Brightness

Color measurement is carried out using a color measuring device called the Toca Color Finder (TCF) by focusing observations on the color that most closely matches the test fish's body color. The color measurement of the test fish is observed by 5 panelists who do not have visual impairments (color blindness or nearsightedness). According to Apriliani *et al*. (2021), observations are made visually by comparing the original color of the fish to the color chart paper (TCF) that has been assigned a value. The image of the TCF is presented in Figure 2. The observation of color brightness begins by taking each fish from its rearing container and then placing it into a measuring glass. The measuring glass is used because it is transparent. Place the TCF paper under the measuring glass, then each panelist observes the brightness of the fish's color and gives a score based on the standardized TCF values. The observed fish are then photographed to document their color changes. Scoring starts from the lowest value of 1, 2, 3 up to the highest of 30, with color gradations ranging from light orange to deep red. Color observations are conducted every 10 days for 30 days.

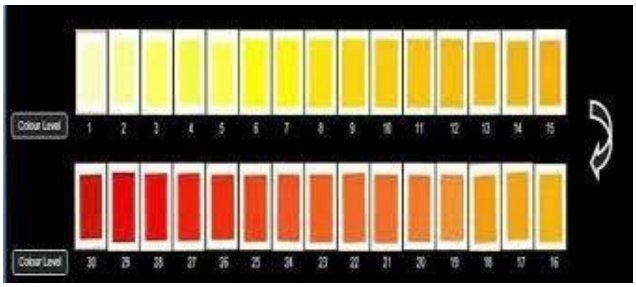


Figure 2. Toca Color Finder (TCF) Color Brightness Measuring Instrument.
(Source:Sitorus *et al*., 2015; Apriliani *et al*., 2021)

The color change value is the result of the assessment given by the panelists. According to Sitompul (2020), the method for determining the final color change value is as follows: each value given by the panelists represents a brightness level, which is then grouped according to each score and recorded based on treatment and replication. The observed value is the value from each treatment and replication. The data analyzed are the changes from the initial color to the final color observed.

5.2. Total Feed Consumption

Total feed consumption (TFC) is the amount of feed consumed, calculated from the total feed provided minus the leftover feed remaining at each feeding, and summed over the maintenance period (Setiawati, 2008; Wicaksana *et al.*, 2015). The TFC is calculated using the following formula (Pereira *et al*., 2007; Mufidah *et al.*, 2017):

$$TFC = F1 - F2$$

where: TFC is the total feed consumption (g); F is the initial feed amount (g); F2 is the amount of leftover feed (g)

5.3. Feed Conversion Ratio

Feed conversion ratio (FCR) is the comparison between the amount of feed consumed and the weight gain of the cultured fish (Lukito and Prayugo, 2007). The FCR is used to calculate feed efficiency, where the amount of feed consumed is divided by the difference between the final and initial fish biomass during cultivation. According to Tacon (1987) and Nugroho *et al.* (2021), the formula for calculating the FCR is as follows:

$$FCR = \frac{F}{(Wt - Wo) + D}$$

where: FCR is the feed conversion ratio; F is the amount of feed given (g); Wt is the final fish weight of the study (g); Wo is the weight of the fish at the beginning of the study (g); D is the weight of the dead fish (g).

5.4. Feed Utilization Efficiency

Feed Utilization Efficiency (FUE) is the percentage of weight gain over a certain period obtained from the amount of feed given each day (Mudjiman, 1989; Wardika et al., 2014). The higher the FUE value, the better the quality of the feed (Afrianto and Liviawaty, 2005). According to Tacon (1987), feed utilization efficiency (FUE) is calculated using the following formula:

$$FUE = \frac{Wt - Wo}{F}\ x\ 100\%$$

where: FUE is the feed utilization efficiency (%); Wt is the final weight of the fish (g); Wo is the initial weight of the fish (g); F is the amount of feed given during maintenance (g).

5.5. Protein Efficiency Ratio

Protein efficiency ratio (PER) is a value that indicates the amount of fish weight produced from each unit of protein in the feed (Tacon, 1995; Rachmawati and Samidjan, 2014). The protein efficiency ratio (PER) is calculated based on the following formula (Zonneveld *et al.*, 1991; Pinandoyo *et al.*, 2020):

$$PER = \frac{Wt - Wo}{Pi}\ x\ 100\%$$

where: PER is the protein efficiency ratio (%); Wt is the fish biomass at the end of the study (g); Wo is the fish biomass at the beginning of the study (g); Pi is the protein content of the feed consumed by fish (%).

5.6. Relative Growth Rate

Relative growth rate (RGR) is the change in weight over a certain period of time (Wibowo *et al*., 2017). According to Muttaqin *et al*. (2016), the growth rate of fish will increase as the amount of food consumed increases. The relative growth rate in this study can be calculated using the following formula (Subandiyono and Hastuti, 2020; Hastuti and Subandiyono, 2022):

$$RGR = \frac{Wt - Wo}{Wo\ x\ t}\ x\ 100\%$$

where: RGR is the relative growth rate (% per day); Wt is the total fish weight at the end of rearing (g); Wo is the total fish weight at the start of rearing (g); t is the tearing period (days).

5.7. Survival Rate

Survival rate (SR) is the percentage of fish that remain alive at the end of the study compared to the number of fish at the start of the rearing period. SR is used to calculate how many fish survive until the end of the study. The survival rate of koi carp is calculated using the following equation (Effendi 1997; Simbolon *et al*., 2021):

$$SR = \frac{Nt}{No} \; x \; 100\%$$

where: SR is the survival rate (%); Nt is the number of fish alive at the end of the study (fish); No is the number of fish at the beginning of the study (fish).

5.8. Water Quality

The water quality parameters observed in the study consisted of temperature, acidity level (pH), and dissolved oxygen (DO). Water quality measurements were carried out daily in the morning at 08:00 am and in the afternoon at 16:00 pm for 30 days using a mercury thermometer for temperature measurement, a pH meter for water pH measurement, and a DO meter to measure dissolved oxygen.

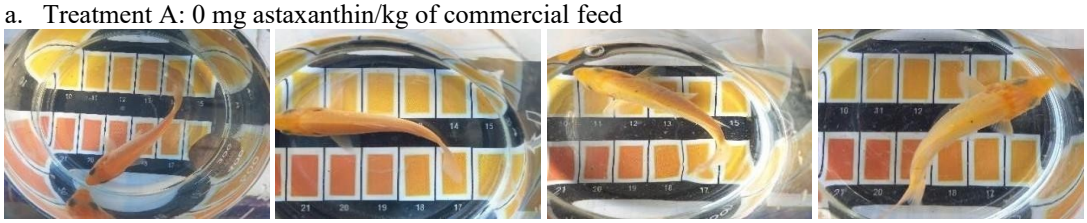
RESULTS AND DISCUSSION

Results

1. Color Brightness

Based on the results of color brightness observations conducted on days 0, 10, 20, and 30, changes in the color brightness of koi fish bodies were observed. The observation results are presented in Figure 3.

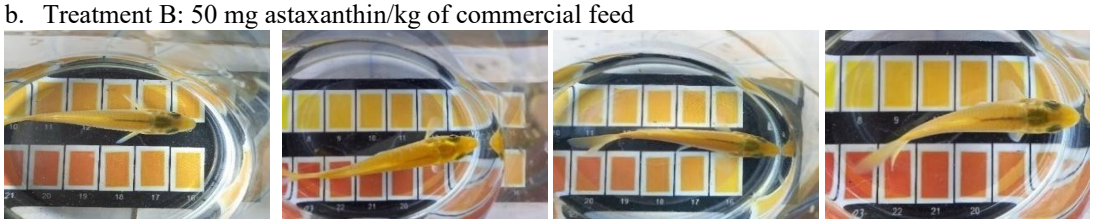
a. Treatment A: 0 mg astaxanthin/kg of commercial feed



Day 0 Day 10 Day 20 Day 30

Description: In the treatment with an astaxanthin dose of 0 mg/kg of commercial feed, the brightness of koi fish showed a decrease in the level of color brightness. From day 0 to day 10 of observation, the color brightness score was at score 3 with an average of 14.7. However, from day 20 to day 30 of observation, the color brightness level dropped to score 3 with a color value of 13.

b. Treatment B: 50 mg astaxanthin/kg of commercial feed



Day 0 Day 10 Day 20 Day 30

Description: In the treatment with a dose of astaxanthin at 50 mg/kg of commercial feed, it was shown that the brightness of the koi fish did not experience either an increase or a decrease in color brightness level. From day 0 to day 30 of observation, the color brightness score remained at score 3, which corresponds to a brightness range of 13-14. Therefore, astaxanthin at a dose of 50 mg/kg has not yet shown any effect on the color brightness level of koi fish.

c. Treatment C: 100 mg astaxanthin/kg of commercial feed

Day 0 Day 10 Day 20 Day 30

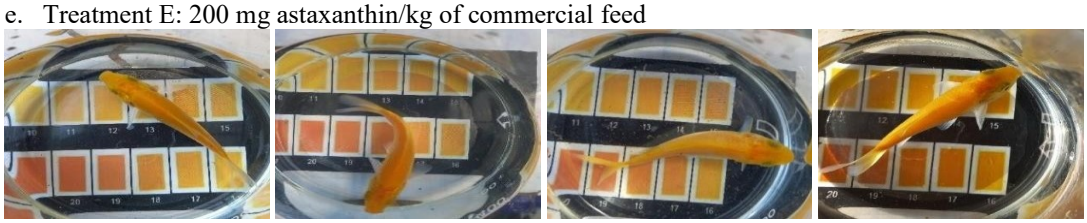
Description: In the treatment with a dose of astaxanthin at 100 mg/kg of commercial feed, it was observed that from day 0 to day 10, the color brightness score remained at score 3 with an average value of 14.3. However, on day 20, the color brightness score decreased to 13.3. By day 30, the color brightness score returned to score 3 with a color value of 15.7. It was observed that the koi's head area showed more intense and vibrant color formation.

d. Treatment D: 150 mg astaxanthin/kg of commercial feed

Day 0 Day 10 Day 20 Day 30

Description: In the treatment with a dose of astaxanthin at 150 mg/kg of commercial feed, it was shown that from day 0 to day 10 of observation, the color brightness score was at score 3 with an average value of 14.3. On day 30 of observation, the color brightness value increased to an average of 15.3. In this treatment, the results of color development during the last 10 days were visible on the koi, particularly in the tail and fins, which became more intense and vivid compared to day 0 of observation.

e. Treatment E: 200 mg astaxanthin/kg of commercial feed



Day 0 Day 10 Day 20 Day 30

Description: In the treatment with an astaxanthin dose of 200 mg/kg of commercial feed, it was shown that from day 0 to day 10 of observation, the color brightness value was 14.7. Observations from day 20 to day 30 showed an increase in the color brightness score to 4, with a brightness value of 16.7. In this treatment, the results of color formation during the last 10 days, as seen in the koi, appeared more vivid and bright on the head, tail, and fins compared to day 0 of observation.

Figure 3. Changes in the Body Color Brightness of Koi Fish on days 0, 10, 20, and 30.

Based on the results of observations of color brightness levels conducted during the study, changes in color and brightness levels were found in koi fish (*Cyprinus carpio*). The best color brightness value was found in treatment E, which is the astaxanthin dose (200 mg/kg), with an average brightness value of 15.83±0.76. Based on the average data of color brightness scores, a histogram can be created as presented in Figure 4.

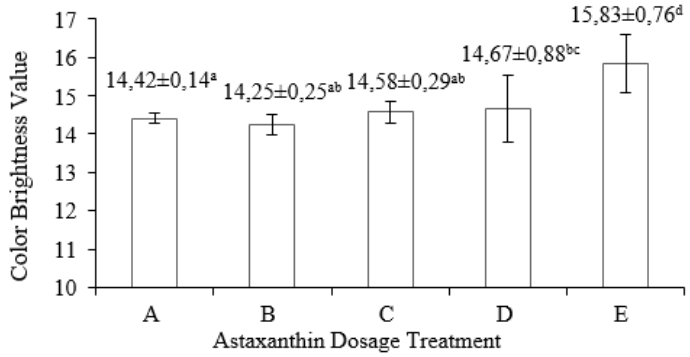


Figure 4. Color Brightness Values of Koi Fish (Cyprinus carpio) after Consuming Feed with Different Astaxanthin Content during the Study.

The analysis of variance showed that the addition of astaxanthin to commercial feed had a significant effect ($P<0.05$) on the color brightness value of koi. Based on the results of Duncan's test, it was found that treatment E was significantly different from all other treatments. Treatment D was not significantly different from treatments C, A, and B, but was significantly different from treatment E. The relationship between observation time and the increase in koi color brightness values is presented in Figure 5.

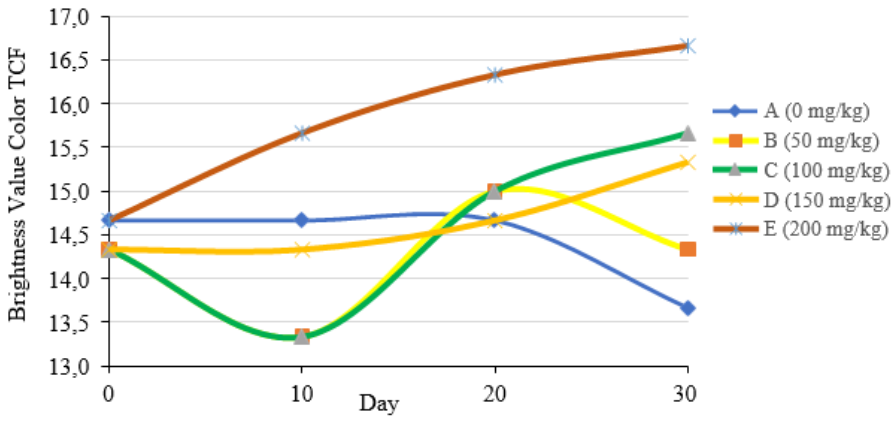


Figure 5. The Relationship between Observation Time and the Increase in Brightness Value of Koi Fish

Based on the curve in the image above, it shows that the time required for koi fish to enhance the brightness of their body color varies with each dose of astaxanthin treatment. On the 10th day of observation, treatments B and C experienced a decrease in brightness value. This is different from treatments D and E, which continued to show an increase in color brightness from the 20th to the 30th day.

2. Total Feed Consumption

Based on the feed consume by koi fish over a period of 30 days, the total feed consumption (TFC) values are presented in Figure 6.

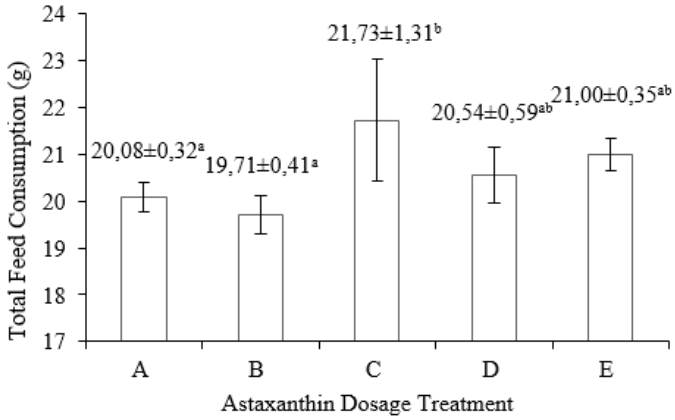


Figure 6. Total Feed Consumption (TFC) of Koi Fish (*Cyprinus carpio*) Fed with Astaxanthin-Containing Feed at Different Dosages During the Study.

Based on Figure 6 above, the highest TFC value was found in treatment C with an astaxanthin dose of 100 mg/kg of commercial feed, with an average of 21.73±1.31 g of feed. Normality, homogeneity, and additivity tests performed on the total feed consumption values indicated that the data were normally distributed, homogeneous, and additive. The analysis of variance (ANOVA) results showed that the addition of astaxanthin to commercial feed had a significant effect ($P<0.05$) on total feed consumption.

3. Feed Conversion Ratio

Based on the total feed consumption of koi fish over a period of 30 days, data on the feed conversion ratio histogram is presented in Figure 7.

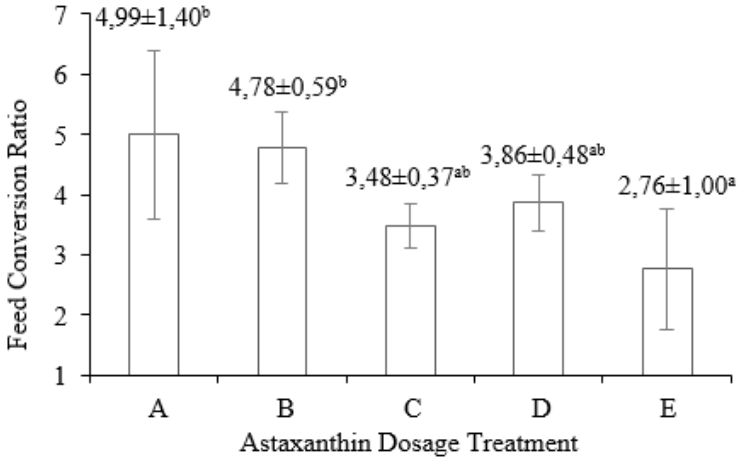


Figure 7. Feed Conversion Ratio of Koi Fish (*Cyprinus carpio*) Consuming Feed Containing Astaxanthin at Different Dosages During the Study.

Based on Figure 7 above, the lowest feed conversion ratio (FCR) value was found in treatment E with an astaxanthin dose of 200 mg/kg feed, with an average of 2.76±1.00 g feed. A low FCR value indicates that it is the best result. Normality, homogeneity, and additivity tests were conducted on the feed conversion ratio values. The test results showed that the data were normally distributed, homogeneous, and additive. Analysis of variance (ANOVA) showed that the addition of astaxanthin to commercial feed had a significant effect ($P<0.05$) on the feed conversion ratio.

4. Feed Utilization Efficiency

Based on the results of the total koi fish feed consumption carried out over 30 days, the feed utilization efficiency values obtained are presented in Figure 8.

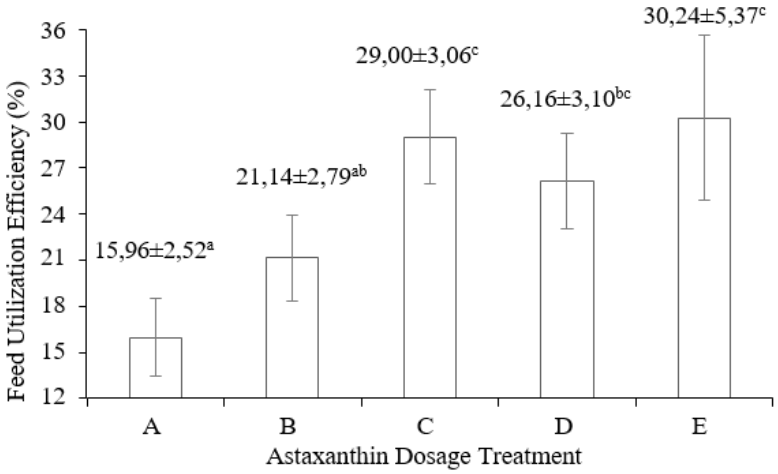


Figure 8. Feed Utilization Efficiency of Koi Fish (*Cyprinus carpio*) Consuming Feed Containing Astaxanthin at Different Dosages During the Study.

Based on Figure 8 above, the highest feed utilization efficiency (FUE) value was observed in treatment E with an astaxanthin dosage of 200 mg/kg, with an average of 30.24±5.37%. The lowest FUE value was found in treatment A, with an average of 15.96±2.52%. Normality, homogeneity, and additivity tests were conducted on the feed utilization efficiency values. The test results showed that the data were normally distributed, homogeneous, and additive. The results of the variance analysis (ANOVA) indicated that the addition of astaxanthin to commercial feed had a significant effect ($P<0.05$) on FUE.

5. Protein Efficiency Ratio

Based on the total feed consumption of koi fish over a period of 30 days, the protein efficiency ratio values obtained are presented in Figure 9.

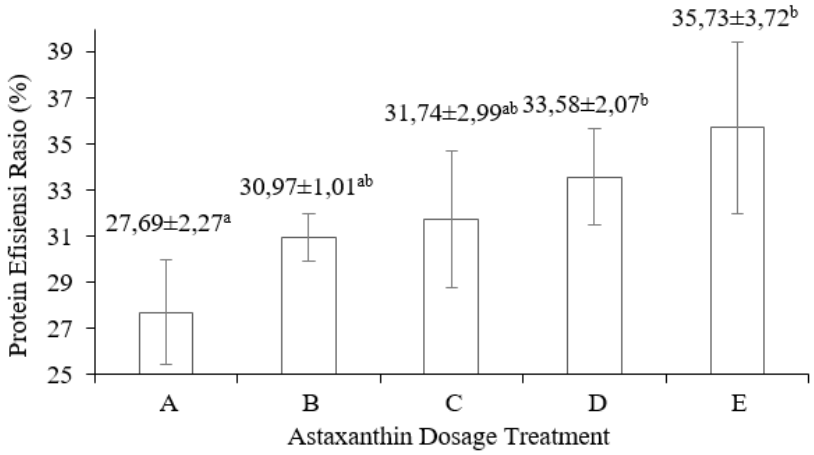


Figure 9. Protein Efficiency Ratio of Koi Fish (*Cyprinus carpio*) Consuming Feed Containing Astaxanthin at Different Dosages During the Study.

Based on Figure 9 above, the highest protein efficiency ratio (PER) value was found in treatment E with an astaxanthin dose of 200 mg/kg, with an average of 35.73±3.27%. The lowest PER value was found in treatment A with an average of 27.69±2.27%. Normality, homogeneity, and additivity tests were conducted on the PER values. The results showed that the data were normally distributed, homogeneous, and additive. The analysis of variance (ANOVA) indicated that the addition of astaxanthin to commercial feed had a significant effect ($P<0.05$) on PER.

6. Relative Growth Rate

Based on the results of the relative growth rate of koi fish conducted over 30 days, the relative growth rate values are presented in Figure 10.

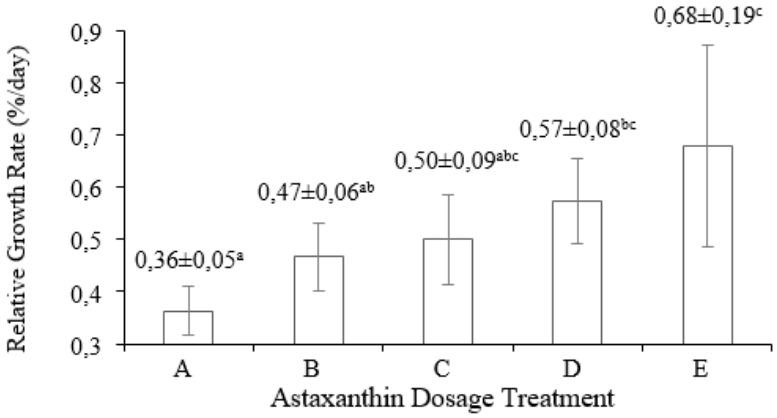


Figure 10. Relative Growth Rate of Koi Fish (*Cyprinus carpio*) Consuming Feed Containing Astaxanthin at Different Dosages During the Study.

Based on Figure 10 above, the highest relative growth rate (RGR) was observed in treatment E with an astaxanthin dose of 200 mg/kg, with an average of 0.68±0.19% per day. The lowest RGR was found in treatment A with an average of 0.36±0.05% per day. Normality, homogeneity, and additivity tests were conducted on the RGR values. The results showed that the data were normally distributed, homogeneous, and additive. The analysis of variance (ANOVA) results indicated that the addition of astaxanthin to commercial feed had a significant effect ($P<0.05$) on RGR.

7. Survival Rate

Based on the results of the survival rate (SR) calculation conducted at the end of the study, data were obtained on the survival rate of koi fish (Cyprinus carpio) that were given astaxanthin in commercial feed. The survival rate values throughout the study are presented in Table 1.

Table 1. Survival Rate of Koi Fish (*Cyprinus carpio*) in Rearing Containers During the Study

Replicate	Survival Rate for Each Treatment (%)				
	A	B	C	D	E
1	66,67	100,00	100,00	100,00	100,00
2	100,00	100,00	100,00	100,00	100,00
3	100,00	100,00	100,00	100,00	66,67

Mean±SD	88,89±19,24	100,00±0,00	100,00±0,00	100,00±0,00	88,89±19,24

Based on Table 1 above, better survival rates (SR) were observed in treatments B, C, and D, with an average SR value of 100%. Normality, homogeneity, and additivity tests were conducted on the survival rates. The test results showed that the data were not normally distributed, not homogeneous, but were additive.

8. Water Quality

Results of water quality parameter measurements, including dissolved oxygen, temperature, and pH. The results of water quality parameter measurements in the koi fish (Cyprinus carpio) rearing media during the study are presented in Table 2.

Table 2. Results of Water Quality Measurements in the Maintenance Media of Koi Fish (*Cyprinus carpio*) During the Study

Treatment	Water Quality Parameters		
	DO (mg/L)	Temperature (ºC)	pH
A	5,3-7,3	26,0-34,5	8,34-9,20
B	5,2-7,1	26,0-35,6	8,38-9,41
C	5,3-7,0	25,5-34,6	8,44-9,04
D	5,4-7,2	25,8-35,5	8,64-9,71
E	5,2-7,1	25,8-34,3	8,43-9,48
Eligibility	>5[a]	25-35[b]	6,5-9,00[c]

Description: [a]SNI (2011); [b]Putri *et al.*, (2022); [c]Nasrullah *et al.,* (2021). The results of water quality parameter measurements during the study showed that the values of these parameters were still within conditions that are suitable and good for use as a rearing medium for koi fish (*C. carpio*), based on literature regarding optimal water quality parameters for the rearing of koi fish (*C. carpio*).

Discussion

1. Color Brightness

The research results showed that the addition of astaxanthin to commercial feed during the study had a significant effect (P<0.05) on the brightness level of koi fish coloration. The best result for astaxanthin addition was found in treatment E, which used a dose of 200 mg/kg of feed and was able to increase the color brightness index by 1 score, raising the initial brightness score from 15 at the start of the study to 18 at the end. A brightness score of 18 indicates that the koi fish's coloration changed from yellow-orange to reddish-orange. In treatment B, even though the fish color did not improve, at the very least the fish were able to maintain the pigment on their bodies during the maintenance period. The changes and improvements in koi fish coloration during the observation period are presented in Figure 3. The decrease in coloration in several treatments was due to the fish experiencing stress during sampling for observation using TCF. On the other hand, the improved color quality was due to the presence of pigment-boosting substances found in the fish feed. The addition of pigment sources to enhance color in the feed will support an increase in the pigment content of the fish's body, or at least enable the fish to maintain its body pigment during the maintenance period (Wayan *et al.,* 2010; Diansyah *et al*., 2019).

The relationship between time and changes in color value showed that treatment A (treatment without astaxanthin) had a constant brightness value from day 10 to day 20, meaning there was no color improvement. However, on the last observation day (day 30), treatment A experienced a decrease in color brightness. Treatment A did not differ much from treatment B, where in treatment B the brightness only increased on day 20 and returned to its initial value by day 30 of maintenance. However, this differed from treatment C, D, and E with astaxanthin doses 100, 150, and 200 mg/kg respectively, which showed increases in brightness values on days 10, 20, and 30. The changes over time for each treatment were caused by the ability of koi fish to synthesize the astaxanthin content in the feed. The higher the astaxanthin content given, the greater the opportunity for increased brightness value.

According to (Bahctiar, 2002; Malini *et al*., 2018), koi fish that are given additional feed containing carotenoids will display an increase in the brightness of their body coloration, and their brightness level will decrease if the supplementation is stopped for a period of time. The process by which astaxanthin affects color brightness begins with the astaxanthin content in the feed flowing into the bloodstream, being stored in fat tissue, and then deposited in chromatophores found in the dermis. The number of pigment cells in the fish's body affects their coloration. If the pigment cells are distributed evenly, the fish's body color will look more intense, but if the pigment is concentrated at a single cell point, the fish's body color will appear pale (Kusuma *et al*., 2012; Khairunnisa *et al*., 2020).

2. Total Feed Consumption

The results of the analysis of variance showed that the addition of astaxanthin to commercial feed had a significant effect (P<0.05) on the total feed consumption of koi fish. The highest feed consumption was observed in treatment C at 21.73±1.31 g, followed by treatment E at 21.00±0.35 g, treatment D at 20.54±0.59 g, treatment A at 20.08±0.32 g, and treatment B at 19.71±0.41 g. The addition of astaxanthin at different doses will also result in different total feed consumption. The high total feed consumption in treatment C may be due to the larger body weight of the fish compared to the other treatments. This is supported by Hadijah *et al*. (2020), who stated that factors influencing feed consumption include body weight, type and quality of feed, growth rate, and feeding frequency. The larger the body size, the higher the energy requirement, which in turn increases feed requirements (Rahmatia *et al.,* 2016). In addition to being affected by fish body weight, the level of feed consumption is also influenced by the quality of the feed content and taste. According to Abidin *et al.* (2015) and Asriyanti *et al.* (2018), differences in feed consumption levels are influenced by components in the feed such as nutrients and protein, as well as feed palatability, which is determined by taste, smell, and color factors that comprise the physical and chemical properties of the feed.

3. Feed Conversion Ratio

The analysis of variance showed that the addition of astaxanthin in commercial feed had a significant effect (P<0.05) on the feed conversion ratio (FCR) of koi fish. The FCR values from lowest to highest were: treatment E at 2.76±1.00 g, treatment C at 3.48±0.37 g, treatment D at 3.86±0.48 g, treatment B at 4.78±0.59 g, and treatment A at 4.99±1.40 g. The FCR value indicates how much feed consumed is converted into fish body biomass, and feed conversion reflects the efficiency of nutrient utilization from the feed by the fish (Mustofa *et al*., 2018). A lower FCR value indicates greater efficiency of fish in utilizing the feed, which will affect overall feed utilization efficiency. The two are closely related; conversely, a higher FCR value indicates that the fish are less efficient in utilizing the feed. Several factors influence the FCR value, including, according to (Schmittows, 1992; Zainuddin *et al*., 2019), the quality and quantity of feed, fish species, fish size, and water quality.

4. Feed Utilization Efficiency

The analysis of variance results showed that the addition of astaxanthin to commercial feed had a significant effect (P<0.05) on the feed utilization efficiency in koi fish. The feed utilization efficiency values from highest to lowest were as follows: treatment E at 30.24±5.37%, treatment D at 26.16±3.10%, treatment C at 29.00±3.06%, treatment B at 21.14±2.79%, and treatment A at 15.96±2.52%. Feed efficiency refers to the fish's ability to utilize the feed provided. According to Sedana and Sumadana (2020), digestibility is directly proportional to feed utilization efficiency, so if the fish has high digestibility, its feed utilization efficiency will also be high. The highest FUE value was found in treatment E, with the addition of astaxanthin at 200 mg/kg of feed. According to Iskandar and Elrifadah (2015) and Dhaja *et al.* (2021), the quality of feed is determined not only by its feed conversion value, but also by its feed utilization efficiency. The addition of astaxanthin at an appropriate dose can help prevent cell damage caused by stress during the process of adapting to environmental changes through feed. This condition will lead to a high level of feed efficiency and relatively high growth as well. The greater the feed efficiency value, the more efficiently the fish utilizes the feed in its body, and the better the quality of the feed (Setiawati *et al.,* 2018).

5. Protein Efficiency Ratio

The analysis of variance results showed that the addition of astaxanthin to commercial feed had a significant effect (P<0.05) on the protein efficiency ratio of koi fish. The values of the protein efficiency ratio from highest to lowest were as follows: treatment E at 35.73±3.27%, treatment D at 33.58±2.07%, treatment C at 31.74±2.99%, treatment B at 30.97±1.01%, and treatment A at 27.69±2.27%. The interaction between feed protein content had a significant effect on feed efficiency values. Treatment feeds with high protein content (Treatment E) resulted in high feed efficiency values as well. Conversely, treatments with low protein content (Treatment A) had lower feed efficiency values compared to the other treatments. This is supported by Fran and Junius (2013), who stated that average feed efficiency increases with increasing feed protein levels, but decreases in feeds with very high protein levels, namely up to 45%. The protein level in feed is influenced by non-protein content, which comes from carbohydrates and fats (Andriyansyah *et al.,* 2020).

6. Relative Growth Rate

The results of the variance analysis showed that the addition of astaxanthin to commercial feed had a significant effect (P<0.05) on the relative growth rate of koi fish. The highest to lowest growth rate values were found in treatment E at 0.68±0.19% per day, treatment D at 0.57±0.08% per day, treatment C at 0.50±0.09% per day, treatment B at 0.47±0.06% per day, and treatment A at 0.36±0.05% per day. The relatively high growth is associated with a high protein efficiency ratio and a high level of feed utilization, resulting in relatively good growth. In addition, growth is strongly influenced by several internal and external factors, including feed, genetics, and water quality. The main factor affecting growth is feed. The protein content in the feed greatly influences growth, as protein functions in forming new tissue for growth and replacing damaged tissue. This is supported by Ridwantara et al. (2019), who stated that the growth rate of fish is strongly influenced by the type and quality of feed given good quality, adequate in quantity, and supported by favorable environmental conditions. The increased growth rate (Treatment E) is thought to be due to the protein content in astaxanthin at the right dosage, so feed

with astaxanthin supplementation can affect the growth rate of koi fish. This is consistent with the findings of Putra et al. (2019), who reported that an optimum astaxanthin concentration of 200 mg was able to produce the best effect on both absolute weight gain and absolute length gain.

7. Survival Rate

Research results on the addition of astaxanthin to commercial feed showed no significant difference in the survival rate of koi fish. The highest survival rate was obtained in treatments B, C, and D, at 100%, meaning that no fish died. Treatments A and E had a survival rate of 88.89%, which is suspected to be due to fish that died because they escaped from the rearing containers. Fish density is very important for comfortable living, as high density causes friction between fish and can lead to death. According to Yani *et al.,* 2019, survival rate is influenced by biotic and abiotic factors. Biotic factors include the age and adaptability of the fish to their environment, while abiotic factors include the availability of food, the quality of the aquatic environment, and biological characteristics.

8. Water Quality

Water quality measurements in koi fish (*Cyprinus carpio*) rearing media over a period of 30 days included parameters such as dissolved oxygen, temperature, and pH. Water quality measurements were taken daily in the morning at 08:00 am and in the afternoon at 16:00 pm. The purpose of these measurements was to ensure that the koi fish rearing media remained in good and optimal condition throughout the research. This is supported by Panggabean *et al.* (2016), who stated that water quality is one of the key factors in the success of fish farming, so that water quality management must meet optimal standards and the living requirements of the fish to support their growth and survival. In general, efforts to manage water quality include water quality monitoring and pollution control. Water quality monitoring covers physical, chemical, and biological parameters (Pradana *et al.*, 2019).

During the study, dissolved oxygen levels ranged from 5.2–7.1 mg/L. These values are considered optimal for koi fish rearing, in line with the SNI (2011) quality standards, which state that the dissolved oxygen content for koi fish maintenance should be >5 mg/L. Fish require oxygen for respiration and to support their metabolic processes, which in turn affects their growth, development, and survival in the rearing media. According to Wahyuddin (2010) and Phonna *et al.* (2022), oxygen levels can be increased, for example, by always providing a flow of incoming water and keeping the water surface open.

The temperature during the research ranged from 25.5–35.5°C. This temperature range is still considered suitable for koi fish rearing. According to Putri et al. (2020), koi fish can still survive in water temperatures between 25–35°C. Temperatures that are too low can affect fish activity, including reduced ability to absorb oxygen (hypoxia), a tendency to remain still in groups, decreased feeding and swimming activity, which can ultimately reduce immunity against disease (Sihombing, 2018).

The pH value during the study ranged from 8.3–9.7. This is considered high but still within an acceptable range for koi fish rearing. According to Nasrullah *et al.* (2021), a good pH value for koi fish maintenance ranges from 6.5–9.0. pH significantly affects aquatic biochemical processes, for example, the nitrification process will stop if the pH is low (Kordi, 2010; Phonna *et al.,* 2020).

CONCLUSION

Based on the results of the study, it can be concluded that: (1) The addition of astaxanthin to commercial feed has a significant effect ($P<0.05$) on color brightness and growth, but does not have a significant effect ($P>0.05$) on the survival rate of koi fish (Cyprinus carpio). (2) The optimal dose of astaxanthin to improve color brightness and growth in koi fish is 200 mg/kg (Treatment E), with an average brightness value of 15.83±0.76, FCR of 2.76±1.00 g, FUE of 30.24±5.37%, PER of 35.73±3.27%, and an RGR value of 0.68±0.19% per day.

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