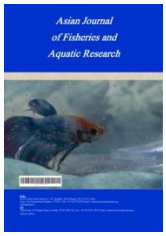

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# The Effect of Differences in the Addition of Astaxanthin and Several Sources of Natural ß-carotene in Increasing Color Intensity of Koi Fish (*Cyprinus carpio* L.)

**Alma Lalita [a*], Walim Lili [a], Iskandar [a] and Fittrie Meyllianawaty Pratiwy [a]**

*[a] Fisheries Department, Faculty of Fisheries and Marine Science, Padjadjaran University, Jl. Raya Bandung-Sumedang, Hegarmanah Jatinangor, Sumedang 45363, West Java, Indonesia.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

This study aimed to examine the effect of differences in the addition of astaxanthin and several natural sources of ß-carotene taken from shrimp head flour and marigold flower flour on the color intensity of koi fish. The research was carried out from January 2022 to March 2022 at the Aquaculture Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University, Indonesia. This study used an experimental method with a completely randomized design (CRD) consisting of four treatments and three replications. The treatments given are control (A), Astaxanthin 0.02% (B), Shrimp Head Flour 10% (C), and Marigold Flower Flour 1.5% (D). Feding of koi fish was carried out for 60 days. Parameters observed included color brightness observed using Toca Color Finder (TCF), survival rate, growth and water quality (temperature, DO, and pH). The results showed that each treatment gave a good effect in increasing the color of koi fish. The addition of astaxanthin 0.02% showed the highest value with an increase in color score of 2.2 on the head and 1.2 on the body. The highest survival rate was in the treatment of adding astaxanthin 0.02% and marigold flower flour 1.5% with a value of 100%. The highest fish growth value was in the addition of shrimp head flour 10% treatment with a weight growth value of 1.938 g and a length growth value of 0.852 cm. The water quality parameter values (temperature: 27-30℃, DO 5.3-7.8 mg/L, and pH: 6.7-7.9) were in normal conditions.

**Corresponding author: Email: almalalita2@gmail.com;*

*Keywords: Koi fish; color brightness; astaxanthin; shrimp head flour; marigold flower flour.*

## 1. INTRODUCTION

Koi fish (*Cyprinus carpio* L.) is one of the ornamental fish that has beautiful color and body shape with high economic value. Koi fish has become a mainstay commodity in several areas in Indonesia, such as Sukabumi, Cianjur, and Blitar because it has succeeded in lifting the community's economy through employment and increasing export value [1].

Color in koi fish is the main reason why they are in great demand by the public as pond or aquarium decorations. The color found in koi fish is caused by the presence of chromatophoric cells or pigment cells found on the outside, inside, and below the dermis on fish scales [2]. Fish cannot synthesize carotenoids in their own bodies. Fish can only synthesize black and white colors, in other words red and yellow cannot be synthesized in their bodies [3]. In the integumentary system of fish, the carotenoid pigments responsible for red, orange, and yellow colors are stored in the xanthophore and erythrophore [4].

Several factors that affect color brightness in koi fish are genetic, environmental and feed nutrition [5]. In addition to the nutritional needs in the manufacture of feed, the addition of ingredients to increase color quality as additional feed is a basic need that has to be considered [6]. The addition of a color enhancing source in fish feed will result an increase in color pigment in the fish's body, at least the fish are able to maintain the color pigment in their body during the maintenance period [2].

Carotenoids are a group of fat-soluble pigments, which increase coloration in many aquatic species [7]. Carotenoids are a group of fat-soluble pigments, which increase coloration in many aquatic species [7]. Astaxanthin (3,3′-dihydroxy-β-carotene-4,4′-dione) is a red xanthophyll carotenoid, which can be converted from other carotenoid precursors in fish diets by major metabolic pathways [8]. Shrimp head flour is one of the wastes produced from shrimp processing which contains carotenoids including β-carotene which can be used in feed for pigmentation [9]. In addition, another source of carotenoids containing β-carotene is marigold flower flour which increase the color of fish [10]. This study was designed to determine the effect of differences in the addition of astaxanthin and some natural sources of β -carotene used, namely shrimp head flour and marigold flower flour which will be added to koi feed and which material has the best effect on koi fish change in body color intensity

## 2. METHODOLOGY

### 2.1 MATERIALS AND METHODS

This research was conducted in the Aquaculture Laboratory, 2nd Building of the Faculty of Fisheries and Marine Sciences, Padjadjaran University, Indonesia. The research was conducted from January 2022 to March 2022. The equipment used in this study were 12 aquariums volume 15 liters of water withsizes (35.9 x 22.0 x 26.2) $cm^3$ with a density of 1 fish/3 L of water, aeration equipment, bowl, digital scales with prescision 0,1 g, Toca Colour Finder (TCF), thermometer, DO meter, pH meter, grinder, and pellet press. The fish used in this study were 60 kohaku koi fish (*Cyprinus carpio* L.) with a weight of 4.21±0.59 g and a length of 6.86±0.30 cm obtained from fish cultivators in the Cimaung area, Bandung Regency, West Java, Indonesia. The method used in this research was an experimental method Complete Randomized Design (CRD) consisting of 4 treatments and 3 replications. The treatments given in the experiment were as follow: (A) Commercial feed without carotenoid (control), (B) Commercial feed with the addition of 0.02% astaxanthin according to [11], (C) Commercial feed with the addition of 10% shrimp head flour according to [12], and (D) Commercial feed with the addition of 1.5% marigold flower flour according to [13]. The process of making feed is carried out by the repelleting method. This method begins with crushing commercial feed using a grinder into flour. Then, mix commercial feed with each ingredient according to the dosage (100 g commercial feed + 0.02 g astaxanthin; 100 g commercial feed + 10 g shrimp head flour; 100 g commercial feed + 1.5 g marigold flower flour). Mixing is done by adding water and then the stirring process is carried out until a moldable dough is formed. The feed is fed into the pellet press to be reshaped into pellets. After that, the drying process was carried out using room temperature. The study conducted for 60 days with artificial feeding treatment with the addition of materials for treatment according to the number of treatments. The frequency of feeding was done three times a day as much as 5% of

the total fish biomass weight per aquarium. Observations of color, fish weight and water quality were made once every 10 days during the research.

## 2.2 Observation Parameters

### 2.2.1 Color brightness

Observations of the level of color change were carried out every ten days for 60 days by three panelists using the Toca Color Finder to avoid bias. Parameters observed were changes in color intensity on the head and body of the koi fish because in both parts of the body there are colors than can be observed. The calculation of the values of fish color change is done by calculating the difference between the average color of the fish on day $0^{th}$ and day $60^{th}$. The color increase in koi fish was observed by comparing the red color on the head and body of the koi fish with the TCF standard which had been assigned a scale of one to seven (Table 1) [14].

### 2.2.2 Survival rate

The survival rate formula used is as follows [15] :

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

Information:
SR : Survival Rate (%)
$N_t$ : Number of live fishes at t the end of study
$N_0$ : Number of live fishes at the beginning of study

### 2.2.3 Absolute Weight growth

The absolute weight growth formula used is as follows [16]:

$$W = W_t - W_0$$

Information:

W : Average weight growth of fish (g)
$W_t$ : Final fish weight growth (g)
$W_0$ : Early fish weight growth (g)

### 2.2.4 Length growth

The length growth formula used is as follows [15]:

$$L = Lt - L0$$

Information:

L : Average length growth (cm)
Lt : Final length growth (cm)
L0 : Initial length growth (cm)

### 2.2.5 Water quality

The water quality parameters observed in this study were temperature measured using thermometer, dissolved oxygen (DO) measured using DO meter, and pH measured using pH meter. Water quality measurements are carried out every 10 days.

## 2.3 Data Analysis

The increase of color quality data was analyzed descriptively and the value of color increasing was analyzed using the Kruskal-Wallis test if there was a difference between treatments, the Z test was performed with a 95% confidence level. Survival rate and growth data were analyzed using analysis of variance (ANOVA) F test with a level of 5% and water quality data were analyzed descriptively comparatively.

## 3. RESULTS AND DISCUSSION

## 3.1 Koi Fish Color Increases

The color of fish skin is basically determined by genetics, but additional ingredients are needed in fish feed to improve color quality because fish cannot produce colors such as red, yellow, green, orange, and blue [17]. The study of color brightness in koi fish of this type was observed in two different body parts, namely the color on the head and the color on the body using TCF and done by three panelists.

Based on the multiple comparisons of the Z test (Table 2), the result of treatment B showed that were significantly different from treatment A but not significantly different from treatments C and D. This indicated that the three treatments had a good effect on increasing koi fish head color. But the highest increase in fish color brightness was found in treatment B with the addition of astaxanthin 0.02% which the score obtained was 2.2±0,269. While the lowest treatment was in treatment A, namely the treatment that did not contain carotenoid sources in the feed. Treatment A has a value of -0.4±0.138, this

indicates a decrease in color on the head of the koi fish. The decrease in color brightness in the control treatment was caused by the commercial feed used did not contain carotenoids so that the fish did not get carotenoids to increase their color. Color changes in fish can be caused by environmental stress such as sunlight, water quality, and pigment content in feed [18]. Fish cannot increase color because they cannot synthesize color in their body [19]. Therefore, in the control treatment, the fish did not get a source of carotenoids from the feed so that the chromatophores did not spread throughout the skin of the fish and caused the color of the fish to turn pale [20].

Based on multiple comparisons with the Z test (Table 3), the result of treatment B showed a significant difference with treatment A. While treatment B did not show a significant difference with treatments C and D. This indicated that the three treatments had a good effect on increasing koi fish body color (Fig 3). But the highest increase in fish color brightness was found in treatment B with the addition of astaxanthin 0.02% which the score obtained was 1.2±0.269. This result showed that astaxanthin can increase color of the head and body of koi fish better than shrimp head flour and marigold flower flour. Astaxanthin is one of the most effective carotenoids added to fish feed to increase fish color brightness because fish will absorb astaxanthin from feed and use it directly as red pigment cells [21]. Astaxanthin which is absorbed by fish from feed and directly used as a red pigment can inhibit the aging process which is obtained naturally in various types of living things [21]. Astaxanthin has antioxidant activity ten times greater than other carotenoids such as zeaxanthin, lutein, cantaxanthin, β-carotene [22].

**Table 1. The TCF color code to be used**

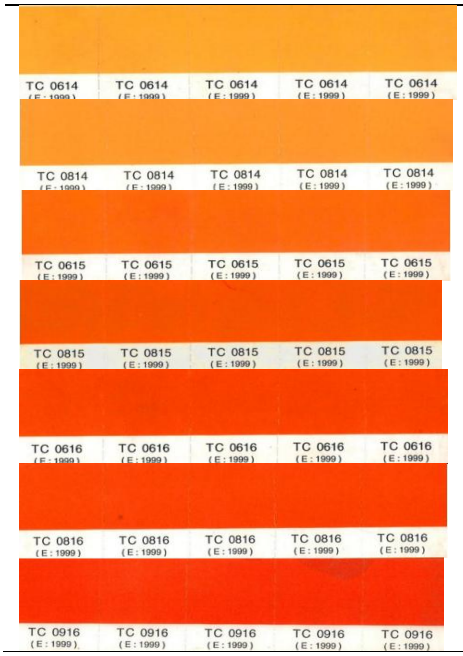
| Color of TCF | Description |
|---|---|
|  | Score 1<br>TCF Code 0614<br><br>Score 2<br>TCF Code 0814<br><br>Score 3<br>TCF Code 0615<br><br>Score 4<br>TCF Code 0815<br><br>Score 5<br>TCF Code 0616<br><br>Score 6<br>TCF Code 0816<br><br>Score 7<br>TCF Code 0916 |



**Table 2. Color value changes in koi fish head day 0th-60th**

| Treatment | 0th Day Value | 60th Day Value | Changes |
|---|---|---|---|
| A (Control) | 3.8±0.42 | 3.3±0.29 | -0.4±0.138[a] |
| B (Astaxanthin 0.02%) | 3.8±0.38 | 6.0±0.27 | 2.2±0.269[b] |
| C (Shrimp Head Flour 10%) | 4.0±0.16 | 5.0±0.10 | 1.1±0.240[ab] |
| D (Marigold Flower Flour 1.5%) | 3.9±0.20 | 4.7±0.19 | 0.8±0.101[ab] |

***Different notations show a noticeable influence P= .05 (Double distance test Z)*

**Table 3. Color value changes in koi fish body day 0th-60th**

| Treatment | 0th Day Value | 60th Day Value | Changes |
|---|---|---|---|
| A (Control) | 2.0±0.30 | 1.6±0.04 | -0.4±0.138[a] |
| B (Astaxanthin 0.02%) | 3.0±0.11 | 4.2±0.17 | 1.2±0.269[b] |
| C (Shrimp Head Flour 10%) | 3.1±0.03 | 3.7±0.13 | 0.6±0.240[ab] |
| D (Marigold Flower Flour 1.5%) | 2.9±0.00 | 3.4±0.20 | 0.4±0.101[ab] |

**Different notations show a noticeable influence P= .05 (Double distance test Z)*

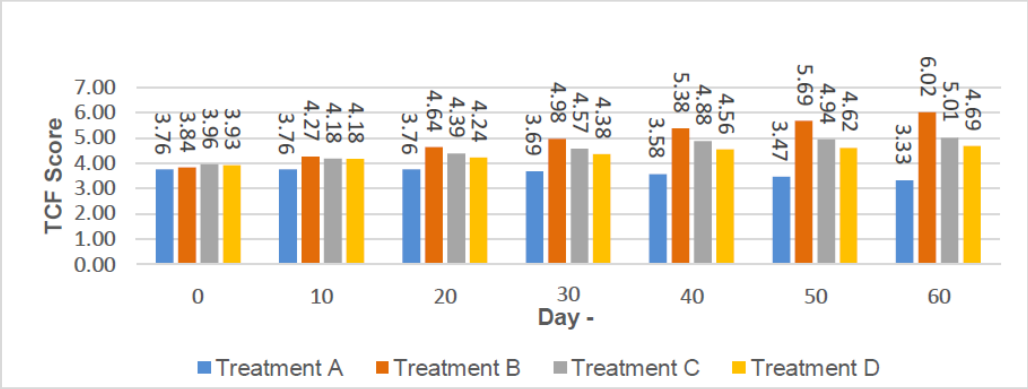



**Fig. 1. Koi fish head color increases**

Based on Fig. 1, it can be seen that there was an increase in color on the 10th day in treatments B (Astaxanthin 0.02%), C (Shrimp Head Flour 10%) and D (Shrimp Head Flour 10%) Marigold interest 1.5%) with scores ranging from 4.18 to 4.27. In the control treatment or without the addition of materials, it was seen that there was no increase in the color value of the koi fish head until day 20 (Fig. 1). On the 30th day there was an increase in red color in each treatment that was added with astaxanthin, shrimp head flour, and marigold flower flour. On the 40th day to the 60th day observations showed that the highest increase in color score was found in treatment B with a score ranging from 5.38 to 6.02 followed by treatments C and D (Fig. 1).

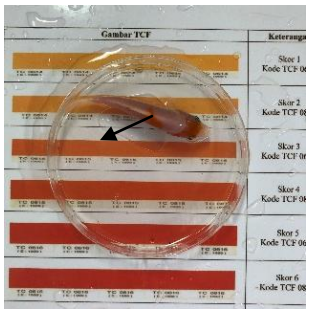
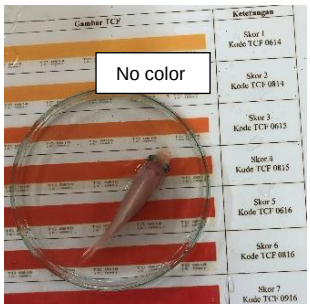
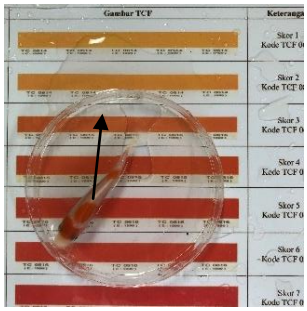
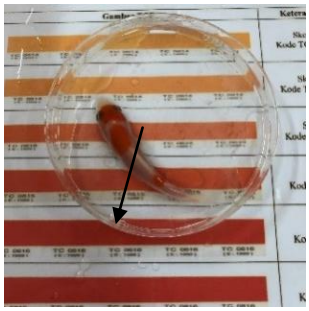
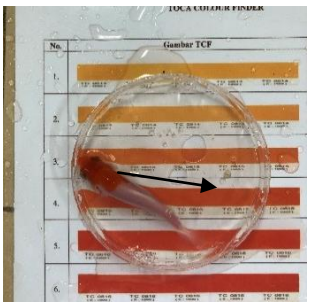
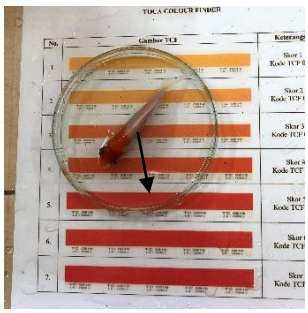
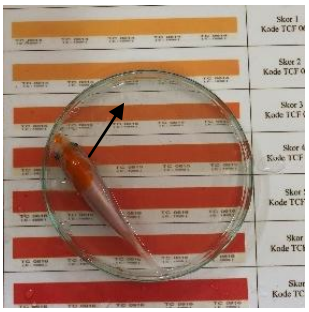
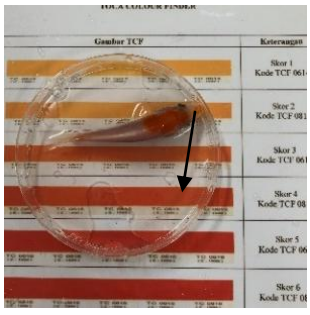
Observation of color changes on the body of koi fish during the study showed an increase in the color score on day 10 to day 60 for treatment B (Astaxanthin 0.02%), C (Shrimp Head Flour 0.02%), and D (Marigold Flour 1.5%). The highest color score increase occurred in treatment B (Astaxanthin 0.02%) with an average color score of 4.22 (Fig. 2). On the 10th to the 20th day there was no increase in color in treatment A, but on the 30th to 60th day there was a decrease in the color of the koi fish body. It is the same with the head of koi fish, which there is a decrease because the commercial feed given does not contain carotenoids so that the chromatophore cells do not spread throughout the fish skin and can cause the color of the fish become pale [20].

Based on the graph of changes in the average color value of the koi fish head and body, treatment C added with shrimp head flour and D added with marigold flower flour had a lower value than treatment B added with astaxanthin. This is because shrimp head flour and marigold flower flour contain crude fiber [23,24]. High crude fiber causes reduced intake of protein and other nutrients that can be digested by the fish body [25]. Fish are less able to digest crude fiber because in the fish intestine there are no microbes that can produce cellulose enzymes so that carbohydrate digestibility in fish is relatively low [26]. Protein and crude fiber content in shrimp head flour is 49.8% and 2.0%, [27]. Meanwhile, the crude protein and crude fiber content in marigold flower flour are 10.17% and 15.13% [24].

In treatment C, the average value of the color looks lower than in treatment B (Fig. 2). This is because the shrimp head flour contains chitin [28]. The chitin content in shrimp head flour is difficult to digest [29]. The chitin content contained in shrimp head flour can inhibit the work of enzymes in digesting fat so that the absorption of carotenoids in the fish body is not

perfect [30]. Carotenoid pigments will be digested better when given in the form of extracts [31]. In treatment D with the addition of marigold flower flour had an average color increase value that was not much different from the treatment with the addition of shrimp head flour (Table 2 & Table 3). However, the mean value of marigold flower meal was slightly lower when compared to the mean value of shrimp head meal (Table 1 & Table 2). This is because marigold flower flour is a source of carotenoids derived from plants that contain high crude fiber. Similar to chitin, crude fiber can also inhibit the absorption of carotenoids contained in marigold flower flour. The crude fiber content in marigold flower flour is 15.13% [24]. The use of crude fiber is not required in fish feed if the levels exceed 10% [32].

**Table 4. Observation of the brightness level of koi fish color**

| Treatment | 0th Day | 60th Day |
|---|---|---|
| **A (Control)** |  **Score 1** |   **No Color** |
| **B (Astaxanthin 0.02%)** |  **Score 3** |  **Score 5** |
| **C (Shrimp Head Flour 10%)** |  **Score 4** |  **Score 5** |
| **D (Marigold Flower Flour 1.5%)** |  **Score 3** |  **Score 4** |

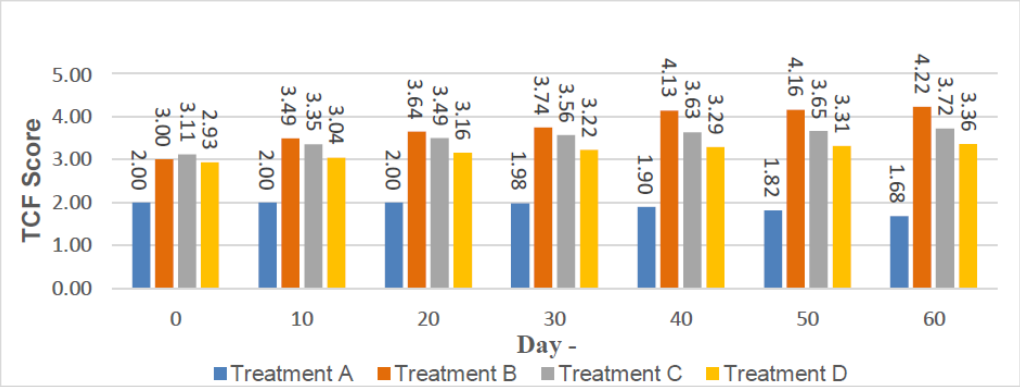



**Fig. 2. Koi fish body color increases**

**Table 5. Average measurement Results of the weight and legth of koi fish**

| Treatment | Absolute Weight Growth (g) | Absolute Length Growth (cm) |
|---|---|---|
| A (control) | 1.667±0.042[b] | 0.817±0.090[a] |
| B (Astaxanthin 0,02%) | 1.603±0.192[b] | 0.807±0.122[a] |
| C (Shrimp Head Flour 10%) | 1.938±0.168[c] | 0.852±0.110[a] |
| D (Marigold Flower Flour 1,5%) | 1.321±0.265[a] | 0.727±0.070[a] |

**Different notations show a noticeable influence P= .05 (ANOVA Test)*

## 3.2 Koi Fish Growth

Growth is defined as a measurable change in tissue size and composition which can represent one of the most important parameters in aquaculture [33]. The growth of koi fish observed in this study was absolute weight and length.

Based on Table 5, the absolute weight growth showed the highest results, namely in treatment C of 1.938 g, followed by treatment A, B, and D with values of 1.667 g, 1.603 g, and 1.321 g. While there is no a significant effect on the absolute length growth of koi fish. The highest treatment of the absolute weight growth was in treatment C with the addition of shrimp head flour. This result showed because shrimp head flour is included in the main protein source which is usually used as an ingredient in making feed. Shrimp head flour contains high protein so that the fish in treatment C got a higher protein intake than the other treatments. Protein content in shrimp head flour is 58.96% [34].

Whereas in treatment A which the feed did not contain carotenoids and treatment B which the feed was added astaxanthin showed no significant difference between the two treatments. This is because the treatment with the addition of astaxanthin did not have a significant effect on the growth of koi fish weight. These results are in agreement with [35] which in his research showed that fish feed added with carotenoids (astaxanthin and cantaxanthin) with feed not added carotenoids had no difference in terms of growth. Treatment D with the addition of marigold flower flour became the treatment with the lowest absolute weight growth value compared to other treatments. This is because the added ingredients, namely marigold flower flour derived from plants, contain high crude fiber. Feed that contain crude fiber can slow down the growth because the feed is difficult to digest [36].

## 3.3 Survival Rate

Based on the results of observations, the highest survival rate during this study was in treatments B and D, which was 100%, followed by treatment C at 93.3%, and treatment A had the lowest survival rate, which was 80%. Death in treatments A and C occured in the first week of treatments. This is because the fish have not adapted to their new environment after being transferred from the fiber tank to the rearing medium. Changes both external and internal can cause stress to fish. External changes that can cause a stress response include temperature fluctuations, lack of oxygen, and transportation time [37]. Significant temperature changes will

**Table 6. Water quality measurements**

| Treatments | Water Quality Parameter Range | | |
|---|---|---|---|
| | Temperature (℃) | DO (mg/L) | pH |
| A | 27– 30 | 5.3 – 7.8 | 6.7 – 7.8 |
| B | 27.1 – 30 | 5.5 – 7.6 | 6.8 – 7.8 |
| C | 27.1 – 29.3 | 5.8 – 7.2 | 6.8 – 7.9 |
| D | 27 – 30 | 5.3 – 7.2 | 6.8 – 7.9 |

make fish difficulties in the acclimatization process so that it will affect fish in carrying out their activities and cause death due to failure in the acclimatization process [37].

### 3.4 Water Quality

Water quality measurements aims to see some parameters are still within tolerance and support the survival of koi fish. Water quality measurements in this study included measurements of temperature, dissolved oxygen (DO), and pH which were measured once every 10 days during the study.

Water quality measurements carried out every 10 days during the study showed that the water quality are still within the limits of feasibility. The results of the water temperature measurements were in the range of 27-30℃. The optimal temperature for fish growth is in the range of temperature values of 25-30℃ [38]. The result of DO measurement on koi fish during the study, the lowest was 5.3 mg/L and the highest was 7.8 mg/L. Dissolved oxygen (DO) levels that are good for koi fish are at a minimum value of 5 mg/L [39]. The pH range for each treatment was from 6 to 7.9, which the value was still in the appropriate range for koi fish maintenance. Koi fish can survive with pH values ranging from 6-8 [40]. Each water quality characteristic will affect the survival, reproduction, growth, production, and management of fish [41].

## 4. CONCLUSION

Based on the results of the study, it can be concluded that the addition of astaxanthin, shrimp head flour, and marigold flower flour have a good effect in increasing color intensity of koi fish with the highest score increase in addition of astaxanthin of 0.02%, with a value of 2.2 on the head and 1.2 on the body. The highest survival rate was in the addition of astaxanthin and marigold flower flour, which was 100%. The best growth was found in the addition of shrimp head flour with a weight growth value of 1.938 g and a length growth value of 0.852 cm. The water quality parameter values were in normal conditions (DO: 5.3-7.8 mg/L; pH: 6.7-7.9; temperature: 27-30℃).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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