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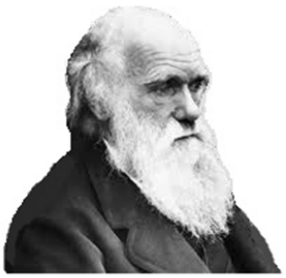

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The Effect of Addition Marigold-Meal to Artificial Feeds for Increasing Color Intensity of Koi Fish (*Cyprinus carpio* Linnaeus, 1758) Strain Kohaku

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ABSTRACT

Feed greatly affects the growth and health of fish, however it also functions to increase the value of the color intensity when specific additional ingredients are added. One of the important ingredients, marigold-meal is one of the natural carotenoid sources. This research aims to determine the optimum concentration of marigold-meal in the artificial feed to increase the intensity color of koi fish, specifically the kohaku strain. The method used was an experimental Completely Randomized Design, consisting of five treatments and four replications. Parameters observed were color intensities analyzed using Kruskal-Wallis analysis, if there were significant differences, Z test would be performed. Research results were the color intensity enhancement of koi fish tails and head until the 40th day showed the highest value in the treatment concentration of 0.0% marigold-meal (positive control) with an average score of 8.00 and 10.00. This value was not different from the treatment concentration of 2.0% marigold-meal and concentration 1.5% marigold-meal. However, this value was not significantly different from concentration of 1.5% marigold-meal. The lowest average score on the tail and head was treatment concentration of 0.0% marigold-meal (negative control). The optimum concentration of the addition of marigold-meal resulting the best color intensity increase was in 1.5% concentration of marigold-meal with the color intensity value of 5.97 in the tail and 6.50 in the head of koi fish Kohaku strain.

***Keywords*:** color, feeds, intensity, koi fish, marigold-meal, *Cyprinus carpio*

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1. INTRODUCTION

Ornamental fish sector has a high potential to contribute upon the economic income of underdeveloped countries, especially in the tropical region [1]. A certain species belonging to freshwater ornamental fish that is much loved because of its beautiful shape, color and pattern was named koi carp which has a quite promising potential. Koi fish has its advantage compared to other ornamental fishes. The price value of koi fish will be greatly determined by the color of ornamental fish since it is one of the important factors that is in great demand [2].

Feed greatly affects the growth and health of fish, while certain additive in feed may function to increase the value of color intensity. Thus, certain additional ingredients were needed in the feed to be able to increase the intensity value of the fish color. Feed containing pigments or certain dyes, such as carotene, may be added in artificial feed hence the effect will be able to increase the amount of pigment in the koi fish, so that the color of fish will be enhanced and be clearer or brighter. Carotenoids are color forming pigments that are found in many types of plants, such as fruits and vegetables [3]. It was reported that pigment from the natural carotenoid sources, such as carrot (*Daucus carota*), marigold petal, China rose petal (*Hibiscus rosasinensis*) and rose petal (*Rosa chinensis*), all they can enhance colour of marine ornamental fish *Amphiprion ocellaris* [4].

Marigold petals in the plant source contain high carotenoid content which is very high in dry weight [5]. Marigold enhanced feed have been used since it contains the product of natural carotenoids which is cheap and easy to obtain and use. Marigolds are a plant that is commonly found in Indonesia and can grow in a normal environment and sufficient sunlight in the tropic regions. The marigold contains various ca- rotenoids, primarily leutin which is the principal component [6]. Therefore this research aims to determine the optimum concentration of marigold-meal in the artificial feed that can increase the intensity color of koi fish (*Cyprinus carpio* L.) strain kohaku.

2. RESEARCH AND METHODS

2. 1. Time and Place of Research

This research was carried out in January 2020 to March 2020. This research took place at the laboratory of Building 4, Faculty of Fisheries and Marine Sciences, Padjadjaran University, Indonesia.

2. 2. Materials and Methods

Equipment used in this research was the aquarium with the size of 30×40×40 cm^3, aeration equipment, Toca Color Finder, digital scales, pelletizer, bowl, thermometer, Dissolved Oxygen (DO) meter, and pH meter. 200 juvenile koi fish (*Cyprinus carpio* L.) Kohaku strain from Sukabumi, West Java, Indonesia, with the size range of 3-5 cm, were used. Commercial feeds with the brand of Matahari was used as a negative control and Agaru brand as a positive control, while marigold-meal with different concentrations was used. Fish was kept in optimum water conditions. The research method used was an experimental method with a Completely Randomized Design (CRD), consisting of 5 treatments and 4 replications. The treatments given in the experiment were as follows: (A) Commercial feed without using marigold meal (negative control), (B) commercial feed with the addition of marigold-meal with a concentration of 1.0%,

(C) commercial feed with the addition of marigold-meal with a concentration of 1.5%, (D) commercial feed with the addition of marigold-meal with a concentration of 2.0%, (E) Commercial feed with color enhanced formula without using marigold meal (positive control). Observation to changes in color intensity was done every 10 days, for 40 days, by observing three fish from each aquarium. Color observation was investigated using the Toca Color Finder, a color identifier. Assessment started from the smallest value of 1 to the biggest score of 10, with gradations of the color intensity from faded orange (TCF Code 0615) to deep red (TCF Code 1117).

Color intensity observations were made on the tail and head of the fish. The measurement of the color intensity of the fish was observed by three panelists who had an understanding of the color of ornamental fish and did not experience visual disturbances (color blindness and farsightedness) and had conducted training in advance. Observations were made visually by comparing the colors of fish with the Toca Color Finder. The results of the data were then analyzed using the Kruskal-Wallis test. If there were significant differences, then Test Z was conducted. Additional data regarding weight gain observation were analyzed using F test with a 95% confidence level to determine the effect of treatment upon the parameters. If the treatment had a significant effect (F count> F table) then it was continued by Duncan's multiple distance test with a 95% confidence level, to find out significant differences. Observation of SR (survival rate) and water quality were analyzed in a comparative descriptive manner.

3. RESULTS AND DISCUSSIONS

3. 1. The Level of the Tail Color Changes from the Koi Fish Kohaku Strain

Based on the research carried out for 40 days, the addition of marigold-meal showed increasing values the color score from koi fish (*Cyprinus carpio L.*) kohaku strain (**Figure 1**).

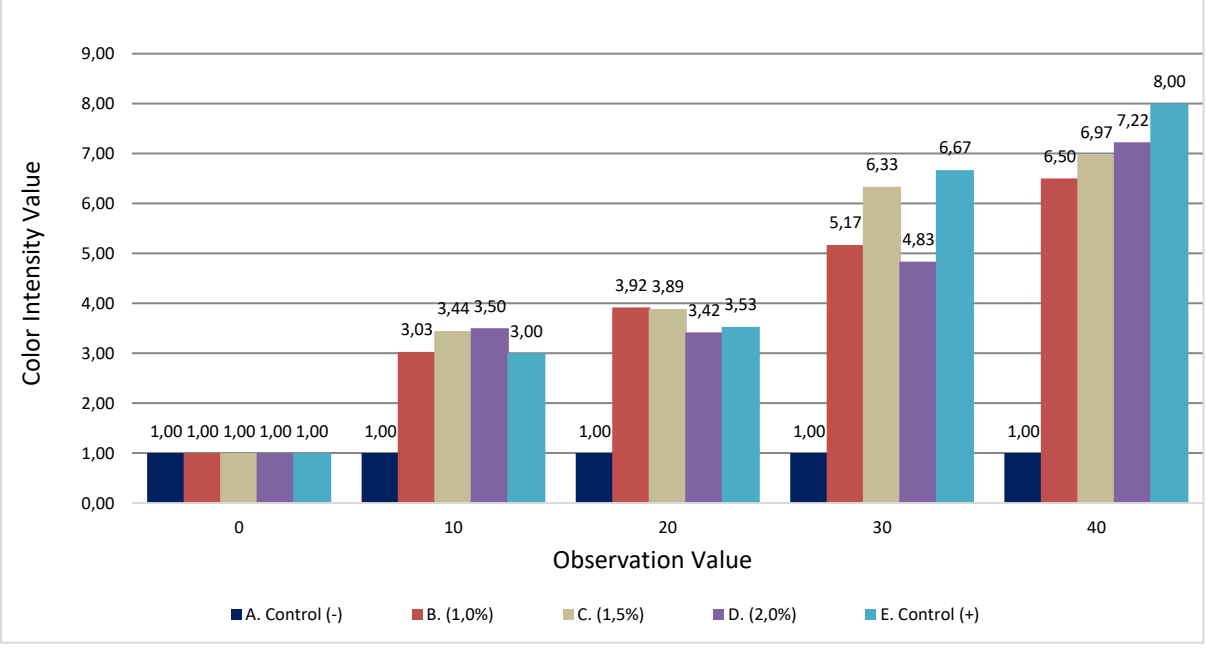
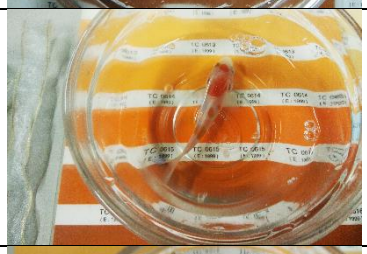
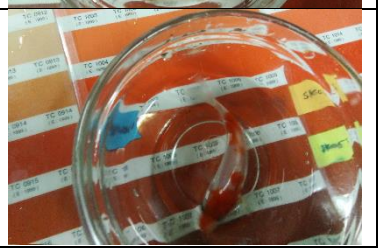


Figure 1. Color Intensity Improvement Charts from the Tail of koi fish kohaku strain from different treatments

The graph in **Figure 1** shows that on the 10th day, an increase in the color values in treatments B, C, D, and E were able to be seen. This shows that the addition of carotenoids contained in marigold-meal can affect the intensity of koi fish. Control treatment without the addition of marigold-meal showed no increase in the color values since this was due to fish that were not fed with the feed containing carotenoid. Normally, chromatophore cells will not spread throughout the skin and will cause a pale-skinned color fish [7].

Table 1. Comparison of the Tail Color Intensisty of Koi Fish During Research

Treatment	Day 0	Day 40
A Negative Control (0%)		
B (1.0%)		
C (1.5%)		
D (2.0%)		
E Positive Control (0%)		

Observation on the 40th day showed the value of an increase in the color intensity of orange in each treatments added marigold-meal (**Table 1**). The highest color score was in the treatment E which was a positive control using a commercial feed, specifically for ornamental fish with an average score 8.00; the second highest was the commercial enhanced with marigold-meal, namely in treatments D and C, with an average scores of 7.22 and 6.97, respectively.

The value of the color intensity score in the treatment B, C, D, and E continued to increase until the 40th day. The results show that the use of color supplements in the form of artificial carotenoid ingredients, mixed with commercial feed, will highly improve the color quality of fish [8].

Table 2. Average Color Intensity Value on the Tail of the koi fish strain kohaku

Treatment	Increase in Color Intensity Value
A. No marigold-meal (negative control)	$0^{a}\pm0.00$
B. Addition of 1.0% marigold-meal	$5.50^{b}\pm0.88$
C. Addition of 1.5% marigold-meal	$5.97^{bc}\pm0.74$
D. Addition of 2.0% marigold-meal	$6.22^{bc}\pm0.63$
E. No marigold-meal (positive control)	$7.00^{c}\pm0.72$

Description: Numbers followed by the same letter notation mean there is no real difference with a 95% confidence level.

Based on the results of the Kruskal-wallis test, it was known that there were significant differences in the control treatment compared to the feed with the addition of marigold-meal (**Table 2**). The results presented in Table 2 show that the highest increase in the color intensity of koi fish occurred in treatment E, namely positive control, however, not significantly different compared to the treatments C and D, with the addition of marigold-meal as much as 1.5% and 2.0%, respectively.

Fish cannot synthesize pigments, they depend on a dietary supply of carotenoids to achieve their natural skin pigmentation and correlate with the market value of ornamental high-value species, such as goldfish [9]. An increase in the positive control (without marigold-meal), was due to the special feed for ornamental containing balanced with the nutrition advantages, such as immunostimulant, high protein, additional vitamins, and minerals needed by ornamental fish. Treatment D (marigold-meal 2.0%) has the highest color intensity value increase compared to the treatment B (marigold-meal 1.0%) but not significantly different from treatment C (marigold-meal 1.5%).

This trend is in accordance with the opinion of ref. [10] which states that the addition of a color-enhancing source in the feed will encourage an increase in color pigment in the body of the fish, or at least be able to maintain the color pigment in the body during the maintenance period.

3. 2. The Level of the Head Color Changes from the Koi Fish Kohaku Strain

Based on research that has been carried out for 40 days, the data result showed an increase in the head color intensity score in koi fish (*Cyprinus carpio* L.) kohaku strain fed with the commercial feed containing different concentrations of marigold.

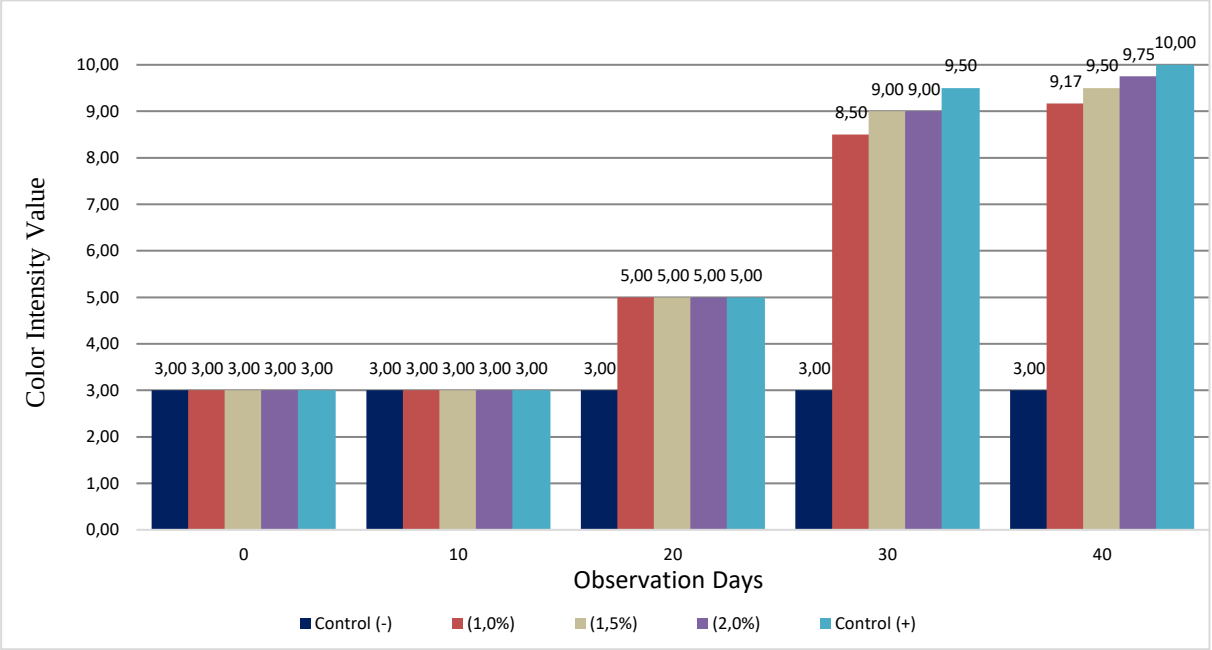


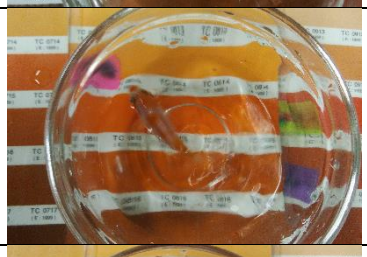
Figure 2. Color Intensity Improvement Charts from the Head of Koi Fish Kohaku Strain from Different Treatments

Observation of color intensity changes made from the head region of koi fish was reported to show an increase in the color intensity score from day 20th until the day 30th. On day 40th the color intensity tended to be stable (**Figure 2**). The increase in the highest color intensity score occurred in the treatment E (positive control with a special commercial feed for ornamental fish) and treatment D with an average color intensity score of 10 and 9.75 at the end of the research. Treatment C had an average color intensity score of 9.50 which was due to the number of carotene sources added to the treatment C which is thought to be appropriate to increase the color intensity of the body of koi fish. Feed containing carotene with the right amount and given regularly will be directly proportional with an increase in fish color intensity [11].

The results of observations on the 40th day (**Table 3**) showed the highest color intensity changes found in the treatment E (positive control) with an average score of 10 and the lowest appeared to be in treatment A (without the addition of marigold-meal) with the average score of 3. The change of color intensity score in treatment C, D, and E continued to increase until the 30th day. This was similar to the part of the fishtail which also had an increase of color intensity value.

Based on the results of the Kruskal-Wallis test, treatment A (negative control without the addition of marigold-meal) was significantly lower compared with all treatments added with marigold-meal and treatment E (positive control, special ornamental fish feed without adding marigold-meal).

Table 3. Figure Comparison of Head Color Intensity of Koi Fish in Early and Late Research

Treatment	Day 0	Day 40
A Negative Control (0%)		
B (1.0%)		
C (1.5%)		
D (2.0%)		
E Positive Control (0%)		

The results presented in **Table 4** show that treatment C with the addition of 1.5% marigold-meal experienced a color intensity increase of 6.50 followed by D treatment with 2.0% marigold-meal at 6.75.

Treatment E was significantly different from C and D but not significantly so that the addition of marigold-meal to the commercial feed was acceptable. Treatments C and D were not significantly different so it can be concluded that treatment C was the best treatment because it was more efficient.

This result shows that ornamental fish business is not enough to only rely on the efforts to spur ornamental fish production, but needs to be accompanied by the efficient measures to improve the appearance of the beauty of color [12]. The improvement in the quality of feed, especially nutrition and the content of raw material sources that have the potential to produce pigments was proven by using marigold-meal.

Absorption and metabolism of koi fish, regarding carotene substances of 1.5% marigold-meal, works optimally because the concentration given is suitable for the ability of the fish to synthesize carotenoids.

Table 4. Average Color Intensity Value on the head of the koi fish strain kohaku.

Treatment	Increase in Color Intensity Value
A. No marigold-meal (negative control)	$0^{a}\pm0.00$
B. Addition of 1.0% marigold-meal	$6.17^{b}\pm0.81$
C. Addition of 1.5% marigold-meal	$6.50^{bc}\pm0.51$
D. Addition of 2.0% marigold-meal	$6.75^{bc}\pm0.44$
E. No marigold-meal (positive control)	$7.00^{c}\pm0.00$

Description: Numbers followed by the same letter notation mean there is no real difference with a 95% confidence level.

3. 3. After Reset

After the 40th day, the treatment (adding marigold-meal to artificial feed) was discontinued and replaced with a negative control feed (Matahari Sakti brand) for the 10th day of maintenance. Further observations were made on the intensity of the color of the koi fish. Disruption of carotenoid enhanced feeding had a significant effect on the color intensity of koi fish which tended to fade but had no significant change. Fish must obtain carotenoids in feed regularly to increase the color intensity [13].

The observations showed that carotenoids in feed were able to significantly increase the color intensity of koi fish (*Cyprinus carpio* L.) kohaku strain. The color intensity of fish produced from feed containing carotenoids was temporary, which indicates that there was a decrease in color quality after carotenoid intake was absent or reduced, but there is no information on the length of time to maintain the color intensity after stopping carotenoid intake [14, 15].

3. 4. Growth

Observation of absolute weight is a supporting parameter observed to determine the effect of marigold-meal added to commercial feed on the growth of koi fish. Growth is an increase in volume and weight at a certain time. Fish growth is closely related to protein availability in the feed. The average growth of absolute fish weight in this study gave different results (**Table 5**).

Table 5. Absolute Weight Growth

Treatment	Average (Gram)
A. No marigold-meal (negative control)	$0.29^{a} \pm 0.24$
B. Addition of 1.0% marigold-meal	$0.95^{b} \pm 0.15$
C. Addition of 1.5% marigold-meal	$1.08^{b} \pm 0.36$
D. Addition of 2.0% marigold-meal	$1.33^{bc} \pm 0.31$
E. No marigold-meal (positive control)	$1.57^{c} \pm 0.27$

The highest weight growth of koi fish during research was in treatment E (positive control of ornamental fish feed) of 1.57 g and D (marigold-meal of 2.0%) of 1.33 g, while the lowest was the treatment A (negative control without an addition marigold-meal) of 0.29 g. The addition of higher carotenoid concentration had an effect on increasing weight growth. This shows that the growth of koi fish kohaku strain was not hampered by the addition of marigold-meal.

Carotenoids do not inhibit the growth of koi fish, but can increase the color intensity of koi fish. These results also show that the addition of carotenoids in the feed was able to increase the nutrient value and also increase the fish appetite, so improved fish growth was observed [16]. Koi fish with the addition of marigold-meal have greater weight growth compared to the negative control fish. Feed added with carotenoids produced a higher growth.

3. 5. Survival Rate

Observation of survival rate showed that the addition of marigold-meal to commercial feed did not give a significant effect on the level of survival rate of koi fish (*Cyprinus carpio* L.) kohaku strain.

Table 6. Survival Rate of Koi Fish (*Cyprinus carpio* L.) Kohaku Strain

Treatment	Survival Rate (%)
A. No marigold-meal (negative control)	100
B. Addition of 1.0% marigold-meal	100
C. Addition of 1.5% marigold-meal	100
D. Addition of 2.0% marigold-meal	100
E. No marigold-meal (positive control)	100

Based on **Table 6**, all treatments had 100% SR. This is presumably because the carotene content in marigold-meal did not endanger fish health. Carotene also naturally functions as a basic ingredient of vitamin A, supports thermoregulation or the process of regulating body temperature hence affecting the fish health [17].

3. 6. Water Quality

Observation of water quality was conducted since it has a large influence upon cultivation. Water quality parameters observed in the study were temperature, DO, and pH. Observation of water quality in the research was conducted every 10 days. The results are presented in **Table 7**.

Table 7. Water Quality Observation Results for Koi Fish (*Cyprinus carpio* L.) Strain Kohaku

Parameter	Results	Reference (SNI 7734-2011)
Temperature (ºC)	24	20-26
DO (ppm)	5.09-5.50	> 5.00
pH	7.31-7.71	6.50-8.00

Temperature observed during the research was maintained at the average of 24 ºC since a thermostat was used in all treatments. Ref. [18] SNI 7734 (2011) asserted that the optimum temperature for the maintenance of koi fish was 20-26 ºC. Dissolved oxygen is the amount of oxygen in milligrams which is contained in a liter of water (ppm).

The observations of DO during the study found that the average DO of each treatment during the study ranged from 5.09 to 5.50 ppm. The dissolved oxygen was still within the tolerance limit for the treatment of koi fish. According to SNI 7734 (2011), the optimal DO for treatment of koi fish is more than 2 ppm.

The results of pH analysis during the study showed that all treatments ranged from 7.31-7.71. The pH value during this study is still within reasonable limits. The measurement results at the beginning of the study showed the pH level was 7.50 (below the optimum limit), however pH experienced variability but it was still considered reasonable (not significant).

Changes in pH was thought to be due to the metabolism of fish during the course of the research. SNI 7734 (2011) described that the optimal pH in the treatment of koi fish ranged from 6.5 to 8.0.

4. CONCLUSIONS

The optimum concentration of adding marigold-meal with the best color intensity increase was in the treatment with 1.5% concentration of marigold-meal and an increase in the color intensity value of 5.97 in the tail and 6.50 in the head of koi fish (*Cyprinus carpio* L.) kohaku strain.

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