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Seagrass Leaf Waste Flour Addition to Feed for Color Pigment Enhancement of Koi Fish (*Cyprinus rubrofuscus*)

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Abstract

The quality of the color determines the price of the ornamental fish, but the color will fade when maintained in an aquarium container. This study aimed to determine the color pigment content of koi fish (*Cyprinus rubrofuscus*) after being given additional feed from seagrass leaf waste flour *Thalassia hemprichii*. This research was conducted for 28 days. This study used a completely randomized design (RAL) with 5 treatments and 3 replicates. The treatment in this study was P1 (control), P2 (addition of 3% seagrass leaf waste flour), P3 (addition of 6% seagrass leaf waste flour), P4 (addition of 9% seagrass leaf waste flour), and P5 (addition of 12% seagrass leaf waste flour). The results showed that the value of color pigment content in all treatments were P1 21.56 μmol/g, P2 22.75 μmol/g, P3 23.49 μmol/g, P4 22.08 μmol/g, and P5 25.76 μmol/g. For absolute growth in all treatments, P1 was 1.37 g, P2 1.53 g, P3 1.80 g, P4 2.73, and P5 3.70 g. For absolute growth in all treatments, P1 was 53%, P2 67%, P3 67%, P4 80% and P5 87%. The survival rate of ornamental Koi (*C. rubrofuscus*) in all treatments was P1 53%, P2 67%, P3 67%, P4 80%, and P5 87%. This research concludes that the color pigment content of ornamental Koi (*C. rubrofuscus*) increases after feeding additional doses of *T. hemprichii,* and the best color pigment increase is shown with the addition of a 12% dose of *T. hemprichii*, namely 25.76 μmol/g.

INTRODUCTION

Given its impact on the growth and survival of carp, feed is one of the most critical components of successful carp farming (C. *rubrofuscus*). Has an impact on life's growth and survival. Protein, fat, carbs, vitamins, and minerals are among the components that must be included in fish feed. While protein is necessary for the process of growth, lipids and carbohydrates make up the majority of the diet's energy sources. The goal is to attain a balance between animal protein (animal protein) and plant protein in the feed (Dani *et al.*, 2005).

Other feed ingredients that can be used to prepare feed include seagrass leaf waste, which has a lot of potential as a raw material for fish feed due to its widespread

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distribution in Indonesian waters. Seagrass has several food sources, including protein, carbs, fat, and dietary fiber (Rompas *et al*., 2012). As a result, it is one of the raw materials that can be used in fish feed. According to earlier research by Niron *et al*. (2023), seagrass flour has the potential to be utilized as a raw feed material due to its high carbohydrate content, which makes it a viable substitute supplement. Fluor nutrients included in seagrass flour can speed up growth in species like *Enhalus acoroides*. The seagrass *T. hemprichii* contains bioactive compounds that may be useful, namely carotenoid biopigments of 334.31 μmol/l, which may improve the color quality of fish. Furthermore, seagrass, specifically *T. hemprichii*, has a very high carbohydrate content, making it a viable substitute for bran, an element in feed. Koi fish feed can be made with seagrass added to it to examine the effects of *T. hemprichii* seagrass on the growth and survival of the fish.

The fish will grow more quickly if the meal is of higher quality. As a result, feed consumption in fish farming is a major challenge. Digestibility determines a fish's capacity to eat one of these. One of the nutritional elements that promotes the fish's digestibility is the feed's fiber level. One source of raw materials high in carbs is seagrass. This type of raw material contains non-digestible polysaccharides, which may one day be used as a source of dietary fiber. The question that has been raised is if adding seagrass flour (*T. hemprichii*) to feed at varying percentages may have an impact on the survival and growth of koi fish.

METHODOLOGY

Ethical Approval

No animals were harmed or improperly treated during this research. It was approved during the due diligence session and proposal seminar.

Place and Time

This research involves 28 days of rearing at Bhayangkara Residence Baiti Jannati Block T1 No 3 Sumbawa Regency and analysis of seagrass carotenoids and koi fish color pigment content at the Analytical Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Mataram University.

Research Materials

Tools and materials used in this research include a container box (25 liters), blower (Resun LP100), aerator, the hose for siphoning, scales, scoop, brush, pH meter, thermometer, Dissolved Oxygen meter (Do9100), bucket, koi fish (*C. rubrofuscus*) size 5-7 cm, seagrass *T. hemprichii*, booster progol (500 g), and AKARI feed (PT SDI, Indonesia).

Research Design

This research is an experimental research using a Completely Randomized Design (RAL), which includes 5 levels of treatment and 3 replicates so that a total of 15 experimental units were obtained. total of 15 experimental units. The treatments tested were the addition of *T. hemprichii* seagrass flour with different concentrations in feed, consisting of P1 (Control, without the addition of seagrass flour), P2 (addition of 3% seagrass flour), P3 (addition of 6% seagrass flour), P4 (addition of 9% seagrass flour), and P5 (addition of 12% seagrass flour).

Work Procedure

Preparation of Cultivan Media

The media used is a container box with a size of 25 liters. The container box is first cleaned using cleaning fluid until clean, then filled with water.

Animal Testing

The test animals used in this study were koi fish obtained at an ornamental fish sales place in Lingsar, West Lombok, which were 4-5 cm in size, had active movements, were not deformed, had uniform size, and were in healthy condition. Each aquarium kept as many as 5 fish.

Making Seagrass Flour

Seagrass leaves were taken from the coast of Mutiara, Kaung Island village,

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Sumbawa Regency. The seagrasses taken were seagrasses that have been detached from their main roots; this aims to preserve the seagrass ecosystem. After it is taken, the seagrass is rinsed using running water until clean to remove mud, epiphytic plants, and salt content from seawater. Alt content from seawater. After cleaning, the seagrass is dried in the sun. After the seagrass is dry, it is mashed using a blender until smooth. Furthermore, seagrass is sieved until it gets the desired flour texture.

Preparation of Combination Feed

The preparation of the combination feed is done by mixing the two ingredients, namely commercial feed and seagrass flour, and seagrass flour. Then, the ingredients are weighed according to the treatment. Next, add 10% progol from the total feed made by dissolving it using warm water as much as 35-40% of the total weight of the feed made. which will be useful as an adhesive supplement. Next, the progol solution is sprayed over the commercial feed. Fish feed is then sprinkled with seagrass flour on top of the commercial feed. Floating Seed Fish Feed was then sprayed again with progol solution, then stirred until evenly distributed so that the seagrass flour could adhere well to the feed.

Fish Cultivation

Fish cultivation was carried out for 28 days by feeding the fish 5% of the total weight of the fish. The feeding time is done twice a day, namely in the morning (08.00) and in the afternoon (16.00) WITA. To maintain the water quality, during the maintenance, the fish were pipetted once a day, in the afternoon.

Sampling

Sampling is done to know the weight of the fish during the maintenance. In this study, the measurement of the weight of koi fish was carried out at the end of the study, which aims to determine the increase in absolute weight of koi fish.

Water Quality Management

To maintain water quality during the study, 20% of the total water volume was replaced daily. Water quality measurements were taken every day. Water quality was measured in the form of pH, temperature, and dissolved oxygen.

Research Parameters

Color Pigment

The content of carotenoids was measured using a spectrophotometer at wavelengths of 480, 645, and 663 nm. The calculation of carotenoid pigment content uses the Khairunnisa *et al*. (2020) formula :

Carotenoid (µmol/g) = ((A480 + 0,114 x A663 – 0,638 x A645) x (V x 103)/(112,5 x 0,1 x 10))

Description :

A480 = Absorbance of wavelength 480 mm
A663 = absorbance of wavelength 663 mm
A645 = absorbance of wavelength 645 mm
V = volume of the extract (ml)

Absolute Weight Growth

According to Effendie (2002), weight and length growth can be calculated using the formula length can be calculated using the formula :

W = Wt – Wo

Description :

W = absolute growth (g)
Wt = average weight of fish at the end (g)
Wo = average weight of fish at the beginning (g)

Survival Rate

The formula used to determine the percentage of survival of test fish according to Effendie (2002) is :

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

SR = Survival rate (%)
Nt = final fish count (fish)
No = initial number of fish (fish)

Water Quality

The measured water quality parameters include temperature measured using a thermometer, dissolved oxygen measured using a DO meter, and pH measured using a pH meter.

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Data Analysis

Analysis of variance at a 95% confidence interval was used to examine the collected data. Additional tests, such as the Duncan test, will be administered if a substantial difference is found.

RESULTS AND DISCUSSIONS

Color Pigment

Analysis results show that the highest carotenoid content of koi fish is found in the 12% P5 treatment, namely, 8.59 µmol/g. While the lowest carotenoid content was in the P1 (Control) treatment, namely, 7.19 µmol/g. The results of data analysis using the One-Away Anova test showed that the addition of seagrass leaf waste in feed did not have a significant effect on the level of carotenoid content of koi fish. The carotenoid pigment content of koi fish can be seen in Figure 1.

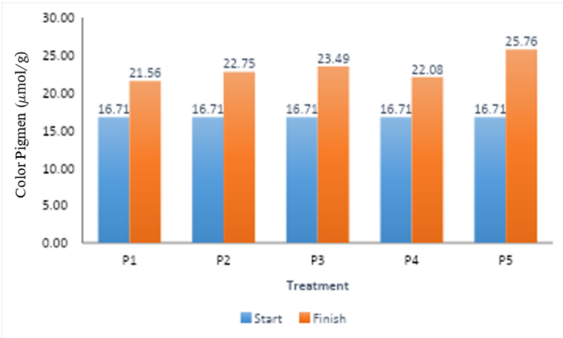

Figure 1. Color Pigment.

Feeding with the addition of seagrass leaf waste gives results that are not significantly different in all treatments. Significantly different in all treatments. Some things that affect the performance of fish color are not only because the fish are unable to accumulate the carotenoids obtained from feed, but also influenced by several factors such as carotenoid content acquisition, the ability of the fish to absorb carotenoids from feed, and others. The difference in color appearance in fish is not only because of the feed factor, but there are other factors such as the level of carotenoid content, the structure of carotenoids in feed, internal factors, and other factors (Sari *et al.*, 2014). Carotenoids in feed, as well as internal and external factors such as size, age of fish, sexual development, and genetic factors.

The increase in color pigments in the fish body will increase when color-enhancing sources are added to the feed. Although the color of the fish does not increase after adding color-enhancing sources, at least the fish can maintain the color pigments in their bodies during the rearing period (Subamia *et al.*, 2010). The observation results showed different carotenoid content in each treatment. The increase in the amount of carotenoids in each fish varies according to the ability of the fish to absorb carotenoids in the feed given. Carotenoids are absorbed into pigment cells through various processes. The spread of pigment cells becomes one of the factors that increase or decrease the color of fish. Following the statement of Noviyanti *et al.* (2015), the process of increasing color intensity begins with carotenoids in the feed, then flowing through the bloodstream and stored in fatty tissue to be deposited in chromatophores contained in the dermis.

The number of pigment cells in the fish's body affects the color of the fish. If the pigment cells are evenly distributed, the color of the fish body will appear more intense, but if the distribution is at one point of the cell nucleus, the body color will be pale. The physiological process of carotenoids in increasing the brightness of fish is consistent with the research of Ahlam *et al.* (2019) that carotenoids are the active

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form of vitamin A. The addition of vitamin A is used for the needs of pigment-forming substances that can brighten the color of koi fish. Mara (2010) added that the process of chemical color formation in the fish body is food containing carotenoids, which in this study the carotenoid content of the *T. hemprichii* seaweed meal used was 334.31 μmol/l, so it enters through the mouth and is digested through the intestines by the enzyme pancreatic lipase and bile salts.

Absolute Length Growth

The highest average absolute growth of koi fish during the maintenance period was found in the P4 treatment with the addition of 9% seagrass flour, which amounted to 5.40 grams. Furthermore, the second highest absolute growth rate was found in treatment P2 with the addition of 3% seagrass flour at 3.83 grams, followed by treatment P5 with the addition of 12% seagrass flour at 3.80 grams, and the lowest growth rate was found in treatment P1 (control), without the addition of seagrass flour at 2.97 grams. The average growth rate of goldfish during the 15-day rearing period is presented in Figure 2.

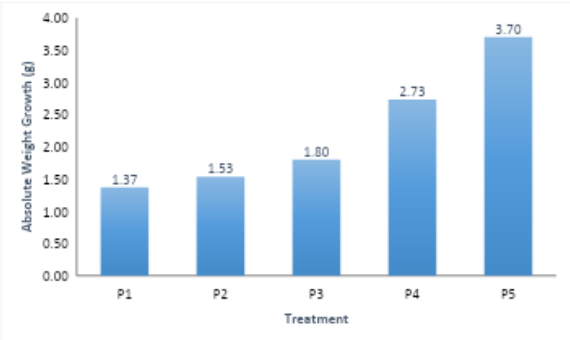

Figure 2. Absolute Weight Growth.

The results of the analysis of variance (ANOVA) showed that the p-value <0.05, then the addition of seagrass flour in the feed did not have a significant effect on the absolute growth of koi fish. Feed is one of the most important factors in aquaculture. Feeding in aquaculture activities needs to pay attention to the nutritional needs of cultivated organisms. The main feed used in this study is Akari, produced by PT Central Windu Sejati. The protein content in this main feed is quite high at 40%.

The difference between the initial biomass and the biomass at the end of rearing is called absolute growth. Many factors, including internal and external factors, affect growth. Internal factors include heredity, age, and ability to utilize food. External factors include water quality, feed, and space (Gusrina, 2008). The results showed that feeding with the addition of seagrass leaf flour showed the highest growth than without the addition of seagrass flour. This is thought to be due to the high content of carbohydrates in seagrass leaves *T. hemprichii*. This is under what was reported by Badui (2010), the high nutritional content of seagrass leaves is 59.26% and the protein and fat content is 5.65% and 0.76%, respectively, all of which are the needs of fish survival. Damayati *et al*. (2016) reported that seagrass leaves can partially substitute the use of mackerel in making fish cake.

Protein is one of the essential needs to fuel the body, besides fat and carbohydrates; it also plays a role in other functions, such as building and regulating substances (Minsas *et al*., 2023). Diachanty (2018) added that protein is a source of amino acids containing the elements carbon (C), hydrogen (H), oxygen (O), and nitrogen (N), and some of them contain molecules of phosphorus (P), sulfur (S) and iron (Fe) and copper (Cu). In line with Kaya's research (2017), the protein content

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of seagrass leaves from the waters of Samboang Beach, Bulukumba Regency, is 1.2%, higher than the protein content of rhizoma and seagrass seeds from Papuan waters. In marine plants such as *Eucheuma cottonii*, which is also one of the habitats of seagrass ecosystems, the protein content fluctuates between 0.61-4.16% from different cultivation depths (Safia *et al*., 2020).

This shows that protein levels depend on the movement of currents and the intensity of sunlight that can enter the marine biota ecosystem. Currents will carry nutrients needed for photosynthesis, and if there is sufficient sunlight exposure during photosynthesis, carbohydrates and protein precursor compounds will be made (Chrismadha *et al*., 2006). According to Coria-Monter and Durán-Campos (2015), the crude protein value of seagrass was 13.8%. The high value of protein is closely related to the bioactive components contained in seagrass. Growth is closely related to the ability of fish to absorb food. This is under the statement of Padang *et al.* (2015) that the success of getting food for consumption will determine the growth of an organism. Besides that, the weight gain of organisms is also influenced by the crude fiber content in feed. Saputri and Mutiarasari (2017) added that another important factor that influences growth is the physiological aspect, which is influenced by the internal condition of fish and the ability to digest and utilize feed for body weight gain. Thus, feeding seagrass leaf waste meal is very beneficial for the growth of cultured koi fish.

Survival Rate

The results of the survival rate research shown in Figure 3 show that the treatment with the addition of seagrass leaf waste is higher than treatment P1 (control). The highest koi fish survival rate is shown in the P5 treatment, which is 87%, and the lowest in the treatment P1 (control), which is 53%.

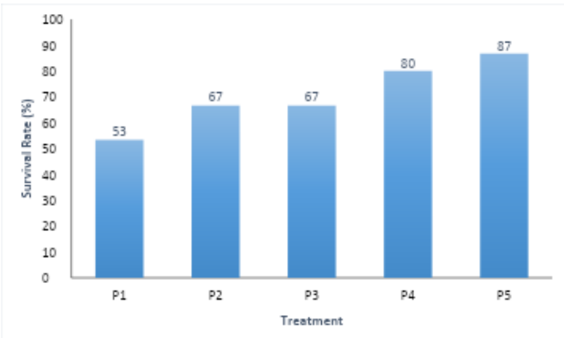

Figure 3. Survival Rate.

The survival rate is the percentage of the number of fish that survive at the end of the study with the number of fish stocked at the beginning of the study (Masitoh *et al.*, 2015). The addition of a combination of seagrass leaf flour showed results that had no significant effect on each treatment given. The survival rate in all treatments was in the range of 53%-87%. Jannah *et al*. (2019) stated that the addition of carotenoids to fish feed produces a good survival rate. It is possible that the addition of seagrass leaves to the feed is not harmful to the survival of koi fish. Carotenoids not only focus on color but can also protect fish from the effects of sunlight and help the oxygen cycle.

In addition, in this study, fish mortality was thought to occur when measuring fish where the fish experienced stress, causing the fish to die. In the P1, P2, P3, P4, and P5 treatments, the survival rate of koi during maintenance was classified as good, this is supported by Rozik *et al.* (2018) which states that the survival rate (SR) ≥ 50% is classified as good, 30-50% survival is moderate and less than 30% is not good. Feeding commercial feed mixed

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with seagrass leaf waste at different doses showed results that were not much different between the five treatments. This shows the high ability of koi fish to live, so the addition of seagrass leaf waste in feed did not significantly affect fish survival during the study. Armiah (2010) states that fish survival is influenced by internal and external factors. Internal factors consist of age and the ability of fish to adapt to new environments and external factors consist of the addition of fish populations in the same space, food shortages, and biological properties associated with capture.

Water Quality

Water quality is an important factor in aquaculture activities. Water quality parameters measured during the study included temperature, pH, and dissolved oxygen content. Data on the results of water quality measurements during 28 days of maintenance can be seen in Table 1.

Table 1 shows that the results of water quality observations made during 28 days of maintenance are still suitable for koi fish farming because there are still underwater quality standards for koi fish farming. The water temperature conditions obtained are still in the range of koi fish maintenance according to (SNI, 2017) to 26-30 ° C. Temperature affects the level of fish appetite and body resistance to disease. Temperature affects the level of fish appetite and body resistance to disease. Low temperatures will affect cell metabolic activity so that it can affect growth, while fish that live or are at relatively high temperatures can result in decreased appetite and feed consumption rates so that the energy obtained is not optimal (Amalia *et al*., 2023).

Table 1. Water Quality.

Parameter	Treatment					Description
	P1	P2	P3	P4	P5	
Temperature (°C)	28	28	28	28	28	SNI (2017), Amalia *et al*. (2023)
pH	7	7	7	7	7	Nirmala *et al.* (2012), Amalia *et al.* (2023)
Dissolved oxygen (mg/l)	5.4	5.4	5.4	5.4	5.4	SNI (2017)

The degree of acidity (pH) in a body of water is one of the chemical parameters to monitor the stability of the water. The pH value obtained in this study ranged from 7.1 to 7.3; the value is still in the normal range of 6.5 to 8 (Amalia *et al.,* 2023). The pH value of a water body can affect the growth of biota in it and can even cause death. Research results Nirmala *et al.* (2012) state that a pH of less than 4 and more than 11 will kill fish, while a pH of less than 6.5 and more than 9.5 within a few hours will affect fish growth.

CONCLUSION

The color pigment content of ornamental koi (*C. rubrofuscus*) fed supplemental doses of seagrass waste meal increased in all treatments, with the best increase in feeding being 25.76 μmol/g with a supplemental dose of 12% seagrass waste meal.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHOR CONTRIBUTION

The contributions of each author are as follows: Muh. Fahruddin analyzed data, drafted the manuscript, and revised it, Davit Aldi collected data; Anita Prihatini Ilyas followed the conception and design experiments.

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