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EFFECTIVENESS OF *Daphnia* sp ENRICHMENT WITH DIFFERENT LEVELS OF *Spirulina platensis* FLOUR ON COLOR BRIGHTNESS AND GROWTH OF KOI CARP (*Cyprinus rubrofuscus*)

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ABSTRACT

Koi (*Cyprinus rubrofuscus*) is one of the freshwater fish farming commodities that has an attractive color. Cultivators are trying to improve the quality of the color of this fish by adding a source of pigment. One is Spirulina, a source of natural pigments added to feed. *Spirulina platensis* has a content of B-carotene (28%) and Zeaxanthin (17%). This study aims to enrich *Daphnia* sp by using *S.platensis* flour to increase koi fish's color brightness and growth. This research was carried out for ±40 days at the Tatelu Freshwater Aquaculture Fisheries Center (BPBAT) Tatelu. The research method is experimental by applying a Complete Random Design (CRD) consisting of four treatments and three replicates. Treatment A (Control), B (3 g/L), C (5 g/L), D (7 g/L) *S.platensis* flour. The test fish used were 96 koi carp seeds with a length of 57 mm and a body weight of 5.47 g. The containers used are 12 aquariums measuring 30×40×40 cm^3 with a volume of 48 L and fish stocking density of 8 fish. The data analysis used was Analysis of Variance, and a further test of the Smallest Real Difference (BNT) was carried out using the SPSS version 26.0 application. The results showed that the application of *Daphnia* sp enriched with *S.platensis* flour could have a real effect on the color brightness and specific growth rate of koi carp ($p<0.05$). The best treatment in this study was the administration of *S.platensis* flour as much as 7 g/L.

Keywords: *Cyprinus rubrofuscus, Daphnia* sp, *Spirulina platensis*

1. INTRODUCTION

Koi (*Cyprinus rubrofuscus*) is derived from carp, Japan's national fish (Kokugyo). This fish is omnivorous (eater of all food) and easily adapts to its environment. Therefore, this fish can be maintained almost everywhere worldwide. Koi fish species that have excellent and stable prices in the world market are Kohaku, Taisho, Sanshoku, Showa, Shiro, Utsuri, Shusui, Asagi, Goromo, Goshiki, Bekko, Tancho, Kinginrin, and Kawarimono[1].

Koi carp is one type of pet that has an attraction to the colors that emerge from its body. Various beautiful colors in the fish body are produced by pigment cells (chromatophores) in the skin or epidermis layer. Chromatophores in the epidermal layer are the color formers in fish. Chromatophores, such as sunlight, water quality, and pigment content in the feed[2], can adjust to the environment. Environmental conditions that have a high thawing intensity will make the fish color brighter and the availability of the proper nutrients in the feed, such as carotenoid content[3]. Environmental factors such as light intensity and the availability of adequate nutrition will make the fish color look brighter[4].

Pigment content in the feed is one factor that affects the brightness of fish color. Efforts to increase the brightness of fish color by adding carotenoids through feed, which are components that form red

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and yellow colors. Astaxanthin added to fish feed is one of the dominant and influential carotenoids that increase the brightness of fish color because fish absorb it from feed and use it directly as a red pigment cell[4]. *S.platensis* is a blue-green algae rich in protein, vitamins, and minerals. *S.platensis* flour contains phycocyanin, chlorophyll, carotene, *β*-carotene (28%), and zeaxanthin (17%)[5]. Carp can absorb carotenoids, β-carotene, and zeaxanthin, primarily in *S.platensis*. Feeding *S.platensis* as supplementary feed is an efficient way to renew the pigmentation process in koi carp. In freshwater, ornamental fish fed *S.platensis* can make the color of the ornamental fish become shiny[6].

Daphnia sp is one of the natural foods favored by ornamental fish. Usually, *Daphnia* sp consumes nutrient particles with a size of about 1 µm to 50 µm. However, particles with a diameter of up to 70 µm can be found in the intestinal contents of large individuals. The dynamics of food absorption by *Daphnia* sp is influenced by the concentration level of a particular food (food concentration), the rate of food uptake in water (feeding rate), and the amount of water filtered per unit of time[7].

Growth is a change in fish, both weight and length and material, in a certain time. Factors that affect fish growth are the protein content of the feed because protein functions to form new tissues for growth and replace damaged tissues. Reduction of protein harms feed consumption, consequently decreasing weight growth. Ambarwati et al.[8] stated that growth is influenced by several factors, namely internal factors and external factors. In contrast, internal factors include heredity, disease resistance, and the ability to utilize food, while external factors are water's physical, chemical, and biological properties. Food and water temperature are the main factors affecting fish growth.

2. RESEARCH METHOD

Time and Place

This research was conducted for ± 40 days, from October 31 to November 30, 2023, at the Tatelu Freshwater Aquaculture Center (BPBAT), Dimembe District, North Minahasa Regency, North Sulawesi Province.

Method

The experimental method is used in this research. The research design was completely randomized (CRD) with four treatments with three replications. The Complete Randomized Design (CRD) model is as follows:

A = *Daphnia* sp without enrichment (control).
B = *Daphnia* sp enriched with *S.platensis* flour 3 g/L
C = 5 g/L *S. platensis* flour
D = 7 g/L *S. platensis* flour

Procedure

Container Preparation

The container for raising Koi fish fry is a glass aquarium measuring 30x40x40 cm^3 with as many as 12 pieces and an aquarium volume of 48 L. The aquariums are placed in a row, and the placement is done according to the research layout. Before use, the aquarium was washed using soap/detergent and then rinsed until completely clean. Next, it was soaked using salt for one day and rinsed with clean water.

After that, the aquarium was filled with water to a height of 20 cm or a water volume of 24 L, aerated, and settled for ± 2 days. The water used as fish-rearing media came from the running water system at the Tatelu Freshwater Aquaculture Center.

Test Fish Preparation

The fish used were koi fry derived from the results of spawning in June 2023 by the koi hatchery team of the Tatelu Freshwater Aquaculture Center, with as many as 120 fish (50-60 mm). Furthermore, a total of 40 seeds (30%) were measured in length (mm) using a caliper (plastic steel caliper 150 mm) and the weight (g) of the seeds using a digital scale (digital scale EP Series, 600 g capacity). As a result, the initial

length of the seeds was 57±4.3 mm, and the initial weight was 5.47±1.3 g.

Before stocking the seeds, the seeds are selected according to the size of each seed and have the characteristics of healthy, non-deformed seeds with sharp patterns and colors, so it can be ascertained that the koi carp studied are healthy and homogeneous. The seed stocking process occurs in the morning at 08.00 WITA when the temperature is stable so the fish do not experience stress.

Nurwahidi[9] stated that a good stocking density for the growth of absolute weight and length of koi fish is 4 fish/12 L. So, this study uses koi fish seeds with a stocking density of 8 fish/24 L or 1 fish/3 L. The seed acclimatization process was carried out for 30 minutes. 96 koi fry were selected from the initial population (120 fish) and placed in each research container. Furthermore, the level of color brightness of koi fry in each treatment container was measured using the Tocca Color Finder (TCF) color indicator, which consists of 30 color levels.

Sampling is done every ten days to determine data on color improvement, length growth rate, and specific weight. Before measuring each variable, the koi fish is soaked using ice cubes with a temperature of $\pm 12^0$ C so that the fish does not experience stress during the color, length growth, and fish weight measurement process.

Daphnia sp Culture and Enrichment Process

Preparation containers used for the enrichment of *Daphnia* sp, namely in the form of 4 buckets as control and treatment containers, and other supporting tools are prepared. Containers and enrichment equipment are washed thoroughly. Enrichment requires materials in the form of 480 g of *Daphnia* sp and 900 g of *S. platensis* flour.

Daphnia sp used in this study came from the culture results at the Tatelu Freshwater Aquaculture Center. Enrichment is done by preparing *S.platensis* flour first, then weighing according to the concentration, namely 3 g/L, 5 g/L, and 7 g/L. After that, *S.platensis* flour was put into a container filled with water with a volume of 1 L/container. Next, stir until the *S.platensis* flour is evenly mixed into the water. Then, enter *Daphnia* sp as much as ± 2 g for each enrichment container.

Enrichment is carried out for 7 hours in each feeding, namely by giving the dose according to the treatment at 00.00 WITA and 10.00 WITA, seed feeding at 07.00 WITA, and 16.00 WITA. The number of *Daphnia* sp given to koi seeds is 0.7 g/aquarium. So, the number of *Daphnia* sp enriched with *Splatensis* flour is 8.5 g/L.

3. RESULT AND DISCUSSION

Color Brightness Level

Observations of the color brightness level of koi were carried out every ten days with measurements four times for 30 days using a tool in the form of M-TCF paper and also observed by 5 (five) panelists who have expertise in seeing fish color because the panelists needed are not color blind.

The results of the enrichment of *Daphnia* sp using *S.platensis* flour show that koi seeds experience a change or increase in color in each treatment. In contrast, in the treatment without enrichment of *S.platensis*, *the* level of color brightness increases from 20.69 to 22.07, while in treatment B with enrichment of 3 g/L *S.platensis* flour, the level of color brightness rises from 20.96 to 23.16, treatment C the level of color brightness rises from 20.71 to 23.38. Treatment D color brightness level increased from 20.64 to 23.78. The results also showed that in each treatment, there was a very significant increase in color brightness due to the influence of *S.platensis* flour consumed by *Daphnia* sp. This can be seen by comparing changes in fish color at the beginning to the end of the study. The observation results during the study showed an increase in color in each treatment, as presented in Figure 1.

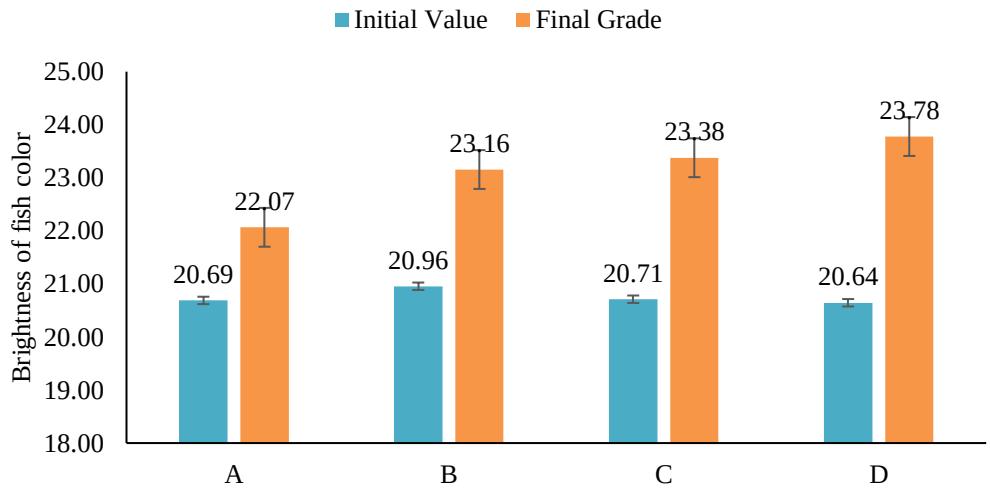

Figure 1. Brightness of the fish's color

Figure 1 shows that the higher the dose of *S.platensis* flour enriched with *Daphnia* sp, the higher the brightness level produced on the koi body. An increase in the brightness of the koi color was found in treatment D with 7 g/L enrichment of *S.platensis* flour compared to treatments A, B, and C. The high pigment compounds influence this in the feed. The high pigment compounds influence this in the feed. Carotenoid pigments are coloring compounds, antioxidants, and vitamin A precursors[10].

Spirulina platensis flour can influence the color intensity in koi because *Spirulina* contains carotenoids that can increase color intensity in fish[11]. This is supported by the opinion of Hadijah et al.[12] that the difference in the level of color brightness in fish occurs due to differences in the number of carotenoids contained in the feed given.

The hormones responsible for the pigmentation process in fish include melanocyte-stimulating hormone (MSH), melanin-concentrating hormone (MCH), and melatonin (MT). MSH is produced in the middle lobe of the pituitary gland, with target cells being chromatophore cells. The primary color of the chromatophore is divided into five cell groups, namely melanophore (black/brown), xanthophores (yellow), erythrophere (red), and leukophere (white)[13].

Fish color is determined by the dominance of pigments in the fat tissue. The higher the content of certain pigments in the fat tissue, the more dominant and brighter the fish color will be[4]. The increase in color brightness in Koi fish occurs due to the presence of ingredients containing carotenoids added to the feed to increase the brightness of the color on the koi's body. This follows the opinion of Indarti et al.[14]; Saiful[10] that the content of carotenoids in the feed influences the increasing number of chromatophore cells. Chromatophore cells are pigment cells that are round and spread throughout the epidermal cell layer of fish skin. Pigment granules that are scattered in the cell cause the cell to absorb light perfectly, so there is an increase in the color of the scales, which causes the color of the scales on the fish to be brighter and more transparent. Fish color is determined by the dominance of pigments in the fatty tissue. The higher the content of certain pigments in the fat tissue, the more dominant and brighter the color of the fish will be.

Adding *Spirulina* sp in feed as a carotenoid source is more influential in improving fish color. However, feeding with the addition of carotenoids has a maximum limit, meaning that if the addition of carotenoids to the feed is excessive, then at some point, it will not provide a high color change but will reduce the color value of the fish.

Giving *Daphnia* sp that has been enriched with *S. platensis* flour affects increasing the brightness of the color on the body of koi ($p<0.05$) due to the content of carotenoids in fish pigment cells (chromophores). The formation of color in the fish body is due to fat-soluble carotenoids that pancreatic lipase enzymes and bile salts will digest in the intestine. The pancreatic lipase enzyme will then hydrolyze triglycerides into monoglycerides and fatty acids[6].

According to Andriani et al.[15], the formation of color in the fish body is due to fat-soluble carotenoids that will be digested in the intestine by the pancreatic lipase enzyme, which will hydrolyze triglycerides into monoglycerides and fatty acids. Bile salts function as fat emulsifiers so that small fat particles called micelle are formed containing fatty acids, monoglycerides, and cholesterol. Carotenoids in the cytoplasm of mucosa cells of the small intestine are broken down into retinol and then absorbed by the intestinal wall along with fatty acids by passive diffusion and combined with micelles, then gather to form bubbles and then absorbed through the lymphatic channels. Furthermore, the micelle and retinol enter the bloodstream and are transported to the liver. Retinol is combined with palmitic acid in the liver and stored as retinyl palmitate. When needed by body cells, retinyl palmitate will be bound by retinol-binding protein (PPR) synthesized in the liver. It is then transferred to other proteins and transported to tissue cells. Thus, carotenoids can be absorbed by the fish's body.

Specific Length Growth Rate

Growth is one of the measurement parameters used to see a material's reaction to the test object's physiological conditions. Internal and external factors strongly influence the growth response itself. In the research, the fish growth response to the treatment did not show significant results or a natural effect on fish growth. According to Silvina et al.[16], fish growth is closely related to protein availability in feed because protein is a source of energy for fish, and protein is a nutrient that fish need for growth. The amount of protein will affect fish growth. The high protein level in feed is influenced by the content of non-protein energy, which comes from carbohydrates and fats.

Observations during the study showed a growth rate of length in each treatment, as presented in Figure 2.

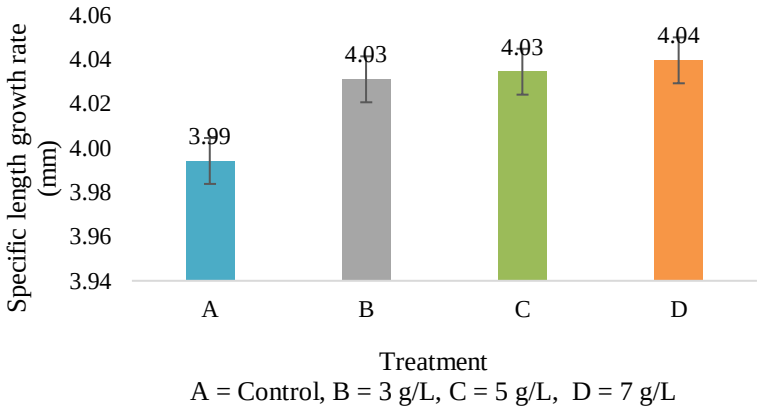

Figure 2. Specific length growth rate

Figure 2 shows the specific length growth rate (%/day) in each treatment, where the highest length gain treatment is in treatment D (7 g/L), which is 4.04 mm/fish, and the lowest length gain is treatment A (Control) which is 3.99 mm/fish. It is suspected that the growth of koi fish is influenced by the ability to respond to and utilize the quantity of feed given[6].

Rosid et al.[17] state that adding *S.platensis* flour to feed affects comet fish's growth length. The best dose of *S. platensis* flour in his research was 2.1 g, which shows that the higher the dose of *S. platensis* flour, the higher the fish length growth. The content of nutrients in the feed also plays a role in the metabolic process in the body of koi fish.

Specific Weight Growth Rate

Observations during the study showed a weight growth rate in each treatment, as presented in Figure 3.

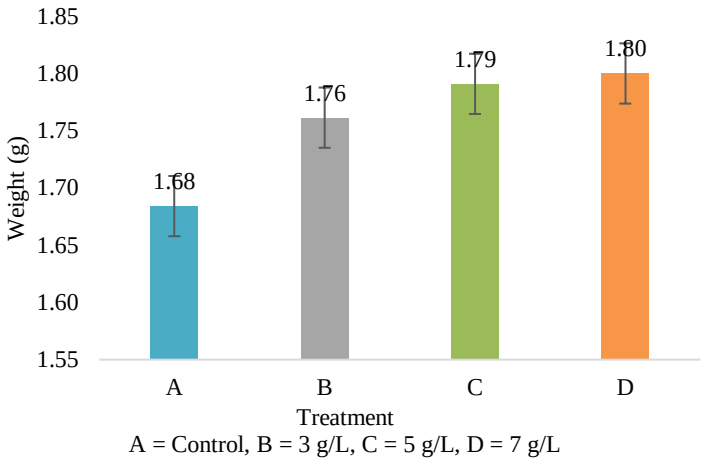

Figure 3. Fish weight growth

Figure 3 shows the specific weight growth rate (%/day) in each treatment, where the highest weight gain treatment is in treatment D (7 g/L), which is 1.80 g/fish, and the lowest length gain is treatment A (Control), which is 1.68 g/fish. This shows that koi can convert the nutrients in the feed into energy. The amount of protein will affect the growth rate of fish. The high protein level in the feed is influenced by the non-protein content, which comes from carbohydrates and fats[6].

During this study, koi's specific weight growth rate (%/day) showed that treatment D provided a high weight growth rate. This condition illustrates that *Daphnia* sp enriched with 7 g *S.platensis* flour provides a reasonable growth rate compared to treatments A, B, and C. This proves that Daphnia sp enriched with *S.platensis* can provide a reasonable growth rate. This proves that *Daphnia* sp enriched with *S.platensis* can offer a high growth rate in koi because the content contained in *Spirulina* in the form of protein 60%, lipids 6% and essential amino acids, including isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. There is vitamin B1, vitamin B2, vitamin B3, vitamin B6, and Vitamin B12. Protein and fat will be digested, absorbed, and metabolized, and after that, they are converted into valuable energy. Fat is a high source of energy in feed. In addition to being a source of energy, it is also a source of essential fatty acids, among others, to maintain the integrity of cell membranes, as a precursor to prostaglandin, prostacyclin, thromboxane, and leukotriene compounds. Fish growth is strongly influenced by the quality of feed and the amount of feed given. The amount of feed given can affect the speed of growth, both weight and length, and the energy obtained from nutritious food[18].

Survival Rate

Survival is expressed as a percentage of the number of fish that live the maintenance period divided by the number of fish stocked, and the survival rate is the opposite of the mortality rate. The percentage of survival of koi fish fry during the 30-day study period can be seen in Figure 4.

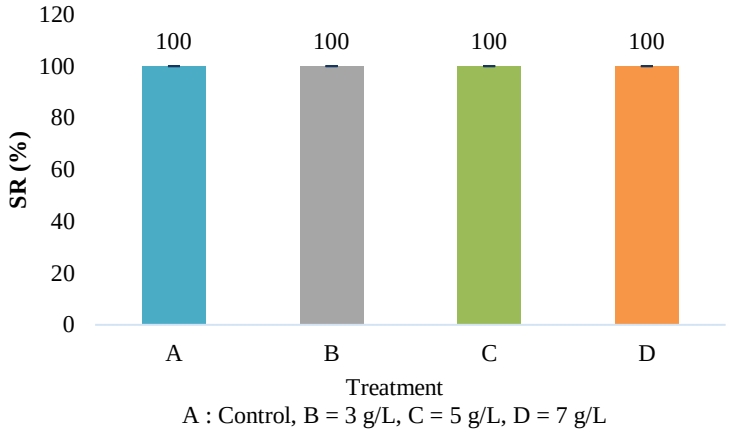

Figure 4. Survival rate of koi

Based on the results of calculations made on the survival rate of koi during the study using different *S. platensis* flour treatments, the survival rate of koi fish reached 100%. The thing that affects the survival rate in each treatment is beta carotene. One of the compounds that can improve fish health is beta-carotene. Betacarotene is a carotenoid compound that has very high vitamin A activity. In the digestive tract, beta-carotene is converted by an enzyme system into retinol, which further functions as vitamin A. Betacarotene and other carotenoids not converted to vitamin A have antioxidant properties to maintain cell integrity in the fish body[10]. In addition, it is suspected that the survival rate of koi fish reached 100% in each treatment influenced by the stocking density of koi is appropriate and the natural food *Daphnia* sp consumed by koi has a relatively high nutritional content including 42.65%, 8% fat, 94.78% moisture content, 2.58% crude fiber, and 4% ash[19].

4. CONCLUSION

Based on the results of the study, it can be concluded as follows: *Daphnia* sp enrichment using *S.platensis* flour has a real effect on the level of color brightness and specific weight growth rate of koi but does not have a real impact in the particular length growth rate of koi. The best dose for color brightness and specific growth rate is in treatment D with 7 g/L *S.platensis* flour.

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