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The effects of *Chaetoceros* sp. meal as a feed supplement on color expression, growth performance and survival rate of discus (*Symphysodon discus*)

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Abstract. Discus fish is one of the ornamental fish that has high economic value based on its color characteristic. To increase fish body color, its need carotenoids obtained from feed. *Chaetoceros* sp. is a natural food rich in carotenoids, especially fucoxanthin. This study aims to evaluate the effect of diets consisting of 0% (control), 5%, 10% and 15% *Chaetoceros* sp. powder on color expression (brightness value and color density), growth performance and survival rates of *Symphysodon discus. Chaetoceros* sp. powder was added into the basal diet. One hundred of discus fish with an average length of 0.5 cm were cultured for 56 days. The result showed that the supplementation of 5-15% *Chaetoceros* sp. powder had no significant effects on the survival rate and the brightness value of discus fish. The increasing of *Chaetoceros* sp. powder supplementation showed a lower rate of growth performance compared to control ($p<0.05$), however the color density was observed to increase significantly at 15% *Chaetoceros* sp. treatment ($p<0.05$). As the conclusion, this study showed that dietary *Chaetoceros* sp. could improve the color expression especially the color density of discus fish and the supplementation level was suggested to be 10% *Chaetoceros* sp powder.

1. Introduction

Discus fish, *Symphysodon discus* belongs to the family Cichlidae originating from calm waters of Amazon River and also known as the King of Aquariums. Discus fish has a potential to be developed in order to meet domestic and export market demands due to its economic value [1,2]. As an ornamental fish, discus fish have many variations of body color including both structural colors and pigmentary. It also known for its beautiful shape and unique variety of patterns [3,4].

Discus fish are considered difficult to cultivate. Discus fish cultivation require special attention in their cultivation techniques because they require good environmental conditions and suitable feed [5]. This fish also very sensitive to changes in the aquatic environment. If the environment is not suitable, the fish become less appetite which will eventually affect growth and can lead to death [6,7]. Beside that, compared to similar species in their natural habitat, cultured fish run into color quality degradation due to stress because inappropriate feeding, handling, and environmental factors [2,8].

An important characteristic in evaluating ornamental fish is the color of the body. In most of ornamental fish, the color that appears comes from the deposition of carotenoids in the tissue and by the presence of chromatophores containing these pigments [9]. Carotenoids are the main source of color in fish skin. Nevertheless, the synthesis of carotenoids cannot be directly performed by fish [10-12]. Fish must obtain optimal levels of carotenoids in the feed to improve their skin color [10,13,14].
The method commonly used by ornamental fish cultivators is to provide natural food such as Tubifex worms, Bloodworms, and *Moina* sp to accelerate growth and brighten colors [15]. Along with the development of fish feed manufacturing technology, sources of carotenoids that were previously only provided in the form of raw materials, now these raw materials can be incorporated into feed [16].
Microalgae are well-known as organisms that contain high-value ingredients such as various nutritional content including polyunsaturated fatty acids (PUFA), pigments such as carotenoids and also other active compounds [17,18]. Microalgae have been widely used to improve color in ornamental fish because they are rich in carotenoids [19]. Application of diets containing 1.52% *Spirullina platensis* enriched with carotenoids and 1% of *Haematococcus pluvialis* for 3 weeks showed an improvement of the color intensity on red velvet sword tail (*Xiphophorus helleri*), rainbow fish (*Pseudomugil furcatus*) and topaz cichlids (*Cichlasoma myrnae* [20]. In discus fish, application of spirulina and astaxanthin was observed to increase survival as well as improve the color intensity of the fish body [21,22]. *Chaetoceros* sp. including marine diatoms that are commonly cultivated and rich in carotenoids, especially fucoxanthin where fucoxanthin production will increase when entering the stationary phase [23-25].

According to [26], the addition of alga oil from *Chaetoceros* sp. on diets compared to other types of algae on Zebra fish *Daniorerio rerio* has the ability to increase total carotenoids and also potentially for fish color enhanchment. In view of the above, this study was conducted to evaluate the effect of diets containing *Chaetoceros* sp. powder on color expression (brightness value and color density), growth performance and survival rates of *S. discus.*

2. Materials and methods

2.1 Fish

Discus fish, *S. discus* were obtained from Research Center for Ornamental Fish Cultivation, Marine and Fisheries Ministry, Depok, Indonesia. *S. discus* with 1,27 cm (0,5 inch) in length were used as many as 360 fish. Fish were acclimatized for a week in an aquarium with environmental conditions similiar to laboratory conditions and fed with a commercial diet.

2.2 Feed Preparation

Basal feed as control consisted of semi-pure raw materials, namely 60% beef heart, 15% ground fish, 15% shrimp, 5% carrot, 2% vitamin premix, 3% spinach. Three diets were designed and *Chaetoceros* sp. powder was added as much as 5%, 10% and 15%, reducing the share of the basal feed. Raw maerials were ground and put into the mold to form 'beef heart hamburger'. The feed is then stored in the freezer.

2.3. Feeding Experiment

The fish were reared in 12 experimental aquariums (50 x 20 x 40 cm^3) that are equipped with aeration. The test feed and control feed were given by applying ad satiation method twice a day (8:00 am and 15:00 pm). Each aquarium was carried out each containing 30 fish. The monitored dissolved oxygen ranged from 6-7 mg L^{-1} through stable aeration, temperature ranged from 28-30 °C, pH 6.5-7.5. The water in the aquarium is siphoned daily in the morning before the first feeding by replacing 1/3 of the existing water. The siphoned water is removed to remove the remaining feed particles. The experiment was carried out at 56 days. The animals were measured the height at the beginning and at the end of study (days 56) and analyzed for color expression on days 56 (the end of the study).

2.4. Color Expression Analysis

Discus color expression was measured on the body surface of the total fish from each aquarium at the end of the study. Color measurement was carried out using a CR-400 colorimeter (Konica Minolta, Osaka, Japan), as one of the measuring instruments considered objective in assessing color quality. Color assessment with a colorimeter produces color quality characteristics, namely lightness or brightness, which characterizes light intensity (bright or dark) and chroma or density, which is an expression of the amount of color-forming substance [27]. Discus fish in this study have two types of colors, namely yellow and silver. In this study only focus on the yellow color, therefore the brighter and higher the concentration value, the better the color quality of discus fish.

2.5. Statistical Analysis

Data from this study were analyzed by one way ANOVA followed by post hoc Tukey test to identify significant differences among treatments. Data are expressed as mean standard deviation and all the analysis was conducted by using SPSS 16.0.

3. Results and discussion

3.1. Survival Rate

The survival rate of discus fish during the rearing period ranged from 81.11±8.39 to 97.22±4.81% (Figure 1). The results of the dose ratio of *Chaetoceros* sp. powder showed no effect on survival rate of discus fish ($p>0.05$). In general, the deaths that occurred in all treatments were thought to be due to the nature of discus fish that were sensitive to a disturbance, both changes in water quality and external disturbances [28], but the water quality during maintenance showed a range of values that were still feasible for discus fish. This is due to the efforts that made to improve water quality during the study, such as water changes as much as 65% of the total volume of the aquarium and maintenance carried out in the aquarium container as well as the installation of light bulbs that are turned on continuously so that the temperature remains stable and a recirculation filter is given. This can be expected to minimize toxic metabolic waste and increase oxygen solubility, so that high mortality does not occur until the end of maintenance. In addition, fish deaths are suspected to be due to disturbances outside of treatment or during sampling. This can cause fish to experience stress which ends in death. Discus fish are fish that are very sensitive to disturbances that can startle the fish, be it noise, human traffic, or sounds and movements that cause stress to the fish. In the treatment with a concentration ratio of 15% and 10%, there were some discus fish that were aloof and had no appetite, were not aggressive and were black all over their bodies. This indicates that the fish are under stress. [29] states that changes in food composition will result in disruption of physiological processes and fish behavior which in turn can reduce fish health and physiological conditions resulting in decreased food utilization, growth, and survival. Seen in the highest percentage ratio, *Chaetoceros* content tends to have more deaths than other treatments.

3.2. Growth Performance

The length growth of discus fish during the rearing period ranged from 0.8±0.08 to 0.96 ±0.03 cm (Figure 2). The results of the ratio of the composition of the *Chaetoceros* sp. powder affected the length growth of discus fish ($p>0.05$). Based on the results of further tests showed that treatment D (15% concentration was significantly different from the control, while the 10% and 5% treatments were not significantly different from the other treatments). Growth is a change in size, both weight and length in a certain period or time [30]. The growth of discus fish slowed down along with the increase in the percentage ratio of *Chaetoceros* sp. powder content. The average increase in fish length for each treatment ranged from 0.80 to 0.96 cm. Based on visual observations, discus fish are voracious fish for food, that is, when the fish are fed it looks very aggressive. However, because fish have a territorial nature (maintain territory), the opportunities for fish to get food become uneven and affect the physiological condition of fish. Discus fish are fish that are difficult to cultivate because they are vulnerable to an environment that is less conducive to life which causes fish to become stressed and

can reduce or even inhibit fish growth [31]. In addition, discus fish also have a level slow growth or relatively long fish growth. In general, it takes ±3 months to reach the market size (2 inches) [32]. Therefore, one of the factors that affect the growth rate of fish is the ability of the fish to utilize the feed.

3.3. Color Expression

The results of color quantification using a colorimeter (Table 1) show that the color brightness value of discus fish ranges from 47.90-50.69% and the density value is between 8.68-16.42%. The brightness value did not have a significant effect on the color of discus fish, but the addition of *Chaetoceros* sp. powder with different doses in the feed gave a significant effect on the color density value ($P<0.05$). The addition of *Chaetoceros* sp. powder feed as a source of carotenoids in the diet affects the color of discus fish compared to the feed that is not added with Chaetoceros both qualitatively and quantitatively, which increases the concentration value by 41-89% (Table 1). This shows that Chaetoceros in the feed is capable or effective to improve the color quality of discus fish. The development of color in ornamental fish is greatly influenced by the coloring pigments produced by carotenoids. According to [26], the highest carotenoid concentration of 0.70 mg/ml was noted in the animal they are fed with the meal mixed with oil obtained from *Chaetoceros* sp., this study claimed that diet could enhanced the coloration in ornamental fishes and become source of carotenoids. Similar to previous reports where the addition of carotenoids in the feed can affect the color of male red cherry shrimp *Neocaridina heteropods* [33], even [34] using synthetic material Carophyll pink with astaxanthin content of 8% as a source of carotenoids in feed was able to improve the color performance of *Lutjanus sebae* red snapper. Another study showed that the supplementation of astaxanthin in the diet of 100-300 mg kg^{-1} and 50-400 mg kg^{-1} could increase color intensity of the sword platy *Xiphophorus helleri* and body pigmentation on discus *Symphysodon* spp respectively [35].

Color quality is related to the amount of pigment in the skin [36]. The quality and presence of color is due to the presence of color cells in the skin called chromatophores. Chromatophores are pigment cells that have a spherical shape and are located spread throughout the epidermal cell layer of fish skin [37]. According to [19] astaxanthin added to fish feed is one of the dominant and effective carotenoids to increase fish color brightness and form red pigment cells. Astaxanthin (red xanthophyll) is applied in aquaculture, pharmaceutical and food industries [38]. so it is suspected that the carotenoid content in *Chaetoceros* sp. powder can influence discus color expression.

Table 1. Color quantification of discus fish feed with different doses of *Chaetoceros* sp. powder

Parameter	Doses of *Chaetoceros* sp. Powder (%)			
	0	5	10	15
Brightness value (%)	50.69±1.33 ^{a}	47.90±1.57 ^{a}	49.34±.51 ^{a}	48.34±1.11 ^{a}
Color density (%)	8.68±0.58 ^{a}	12.26±0.47 ^{b}	15.16±1.17^{bc}	16.42±1.30 ^{c}

Note: Data are mean ± SD. Different letters indicate significant differences ($P < 0,05$)

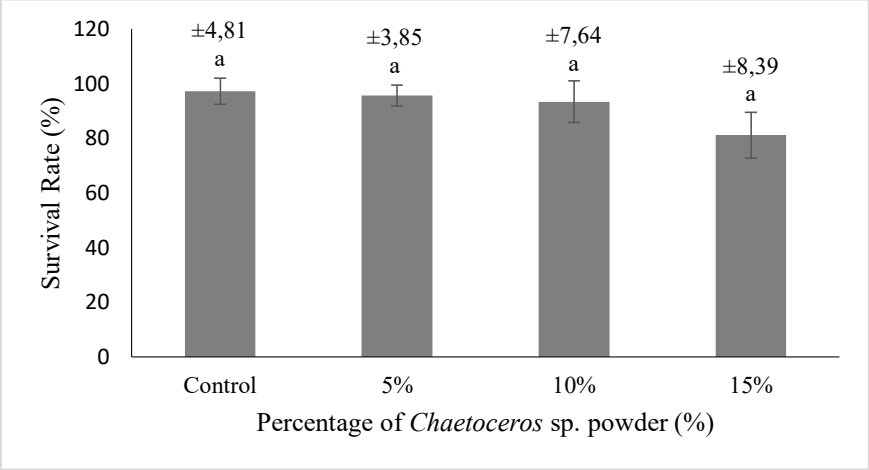

Figure 1. Effects of *Chaetoceros* sp. powder as on survival rate of discus (*Symphysodon discus*) during rearing period. Indicates significant difference (P < 0.05).

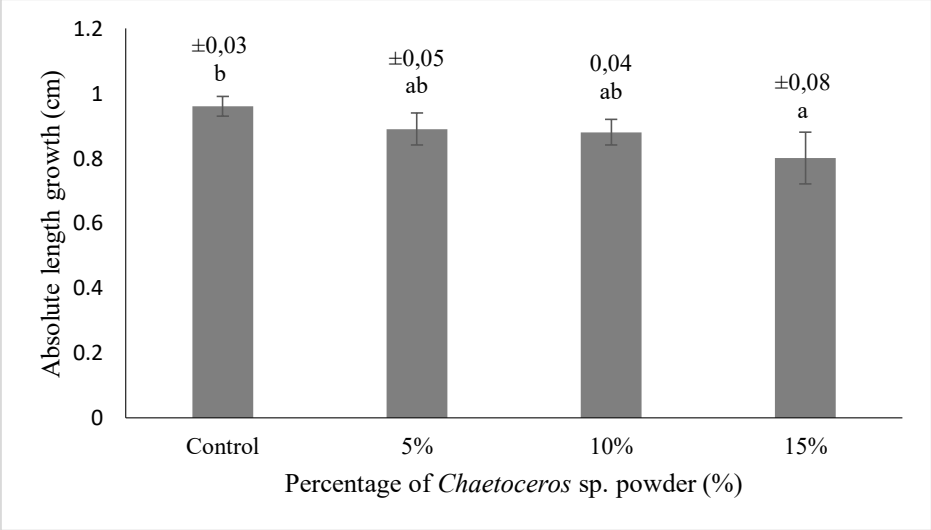

Figure 2. Effects of *Chaetoceros* sp. powder as on growth performance of discus (*Symphysodon discus*) during rearing period.

4. Conclusion

The supplementation of 5-15% *Chaetoceros* sp. powder had no significant effects on the survival rate and the brightness value of discus fish. The increasing of Chaetoceros sp. powder supplementation showed a lower rate of growth performance compared to control ($p<0.05$), however the color density significantly increased, 15% *Chaetoceros* s sp. showed highest color density compared to the other treatments ($p<0.05$). Dietary *Chaetoceros* sp. could improve the color expression especially the color density of discus fish and the supplementation level was suggested to be 10% *Chaetoceros* sp powder.

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