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THE EVALUATION OF *Spirulina platensis* DIETARY SUPPLEMENTATION ON THE GROWTH PERFORMANCE AND COLOR QUALITY OF POLAR BLUE CICHLID (*Archocentrus nigrofasciatus* × *Amphilophus citrinellus*)

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ABSTRACT

This study aimed to evaluate S. platensis powder dietary supplementation on the growth performance and color quality of polar blue cichlids. This study used a completely randomized design experimental method with four different dietary supplementation doses of S. platensis powder, namely 10 g/kg diet (A), 20 g/kg diet (B), 30 g/kg diet (C), and 0 g/kg diet (K). These treatments were replicated four times. The polar blue cichlids as fish samples had 4.07 ± 0.12 cm and 2.15 ± 0.22 g. Diets (38% protein) were produced by coating the commercial feed with S. platensis powder in appropriate dosage. Feeding was performed until apparent satiation twice a day for 45 days. All parameters, including growth performance (feed intake, weight gain, length gain, specific growth rate, feed conversion ratio, survival rate) and color quality (total grid percentage and total chromatophores), were analyzed using an analysis of variance and the Duncan's multiple range test was applied to obtain the best treatment in growth performance and color quality. The dietary supplementation of S. platensis powder had no significant effect on the growth performance of polar blue cichlids. However, this treatment provides a higher total grid percentage of blue color and total chromatophores in the fish. The best treatment on color quality was found in a 30 g/kg diet dose of S. platensis powder. Therefore, 30 g/kg dose of S. platensis powder is effective to improve the color quality of polar blue cichlids, although providing no significant effect on the growth of the fish.

Keywords: ***:*** Cichlid; Color; Growth; Spirulina

INTRODUCTION

Most people are attracted to ornamental fish due to their interesting color gradation and behaviors. In 2020, the ornamental fish production in Indonesia increased by 1.5 million fish, then continued to elevate to 1.6 million fish in 2021 (KKP, 2023). World Bank (2023) reported that demands for ornamental fish have been growing since 2017 at USD 315.12 millions to USD 366.61 millions in 2021. One of potential ornamental fish as an export commodity from Indonesia is a polar blue cichlid (*Archocentrus nigrofasciatus* × *Amphilophus citrinellus*).

The polar blue cichlid is included in the Cichlidae family from the cross-breeding product of female Midas cichlid (*Amphilophus citrinellus*) and male zebra cichlid (*Archocentrus nigrofaciatus*). This fish has many been cultured as an ornamental fish commodity in Indonesia, due to its distinct blue color with black band around its body. This interesting color comes from the chromatophores (pigment cells) in the dermal layer. Luo et al. (2021) reported that chromatophores determined the color intensity on various fish species, whereas high intensity indicates the abundant chromatophores distributed on fish integument. In addition, growth performance also determines how effective and efficient the ornamental fish culture occurs, to produce a qualified ornamental fish with optimal color intensity and high productivity level to fulfill the market demand (Vissio et al., 2021).

Edmond (2022) mentioned that polar blue cichlids are highly adaptable species that can optimally grow at 24 - 28°C and pH of 6.4 – 7.5. In addition to the environment, feed is also one of the factors that affects the color quality and intensity of ornamental fish. Thus, dietary supplementation with pigment properties to regulate the color intensity in ornamental fish is necessary (Sharma, 2020). Pigment dietary supplementation in ornamental fish has been performed using synthetic pigment properties such as carotenoids (Meilisza et al., 2018; El-Gawad et al., 2019; Nakano & Wiegertjes, 2020). However, synthetic pigment will elevate the feed cost due to a complicated production that remains economically ineligible for industrial purpose (Igreja et al., 2021). This condition needs an alternative material such as natural pigments that are frequently available and can be produced sustainably.

A natural pigment source that can be applied to improve the blue color in ornamental fish, mainly polar blue cichlid, is *Spirulina platensis*. *S. platensis* contain phycocyanin and β-carotene (carotenoids) as natural pigments that are considered to improve the color intensity and growth performance in fish, mainly ornamental fish (Rosid et al., 2019). After the extraction process with sodium phosphate buffer, the phycocyanin content in *Spirulina* sp. reaches 100-150 mg/g (Pez Jaeschke et al., 2021).

Studies related to the application of *S. platensis* as natural pigments in diets to improve color intensity and growth performance have many conducted in several ornamental fish, namely goldfish (*Carassius auratus*) with 1.2% *Spirulina* supplementation in the diet (Noviyanti et al., 2015), koi fish (*Cyprinus carpio*) with 1% *Spirulina* supplementation in the diet (Utomo et al., 2007), and clownfish (*Amphiprion ocellaris*) with 3% *Spirulina* supplementation in the diet (Hadijah et al., 2020). Nevertheless, an application of *S. platensis* powder dietary supplementation in polar blue cichlids is still limited, which requires a further study to evaluate the effect of *S. platensis* powder through dietary supplementation on the growth performance and color intensity of polar blue cichlids.

RESEARCH METHODS

Period and Location

This study was performed in July-November, 2023 at several laboratories of the Faculty of Agriculture, Tidar University, Magelang, Indonesia. Fish rearing and sampling were performed in the field Laboratory of Bandongan, diet production and proximate analysis were performed in the laboratory of Animal Nutrition, and color intensity analysis was performed in the laboratory of Animal Physiology.

Design

Experimental method was applied in this study through the completely randomized design method. This method used four treatments and four replications. The treatments contained four different dietary supplementation concentrations of *S. platensis* powder, namely 10 g/kg diet (A), 20 g/kg diet (B), 30 g/kg diet (C), and 0 g/kg diet (K).

Procedures

Rearing Media

The rearing media used 12 units of 50 cm × 30 cm × 30 cm aquarium. These rearing media were washed and dried, then filled with water at approximately 75% of the aquarium volume. Furthermore, the aquaria were also equipped with aeration and filter system for oxygen supply and water quality control.

Fish Samples

The polar blue cichlids were obtained from fish culturists in Temanggung, Indonesia. These fish had 4.07 ± 0.12 cm length and 2.15 ± 0.22 g weight. Before stocking, fish were acclimatized for 24 hours in a fish stock container. Fish were randomly stocked at 15 individuals per aquarium. In addition, the remaining fish were preserved as initial samples for body protein and lipid analyses.

Diets

For diet production, commercial feed was used (*Prima Feed PF-1000*, *PT. Matahari Sakti, Surabaya, Indonesia*) with iso-protein content (35.16%). The *S. platensis* powder was also obtained commercially (*PT. Algaepark Indonesia Mandiri*, *Klaten, Indonesia*) as a diet supplement in this study. Diets were produced by mixing the commercial feed with *S. platensis* powder in appropriate dosage (as mentioned in treatments) and 30% of egg whites as a supplement-coating agent, homogeneously. Then, diets produced were dried in an oven at 60°C for 3-4 hours. After heating, diets were kept in a plastic container and some of which were preserved for proximate analysis.

Rearing and Feeding

Fish rearing was performed for 45 days to observe the growth performance and color intensity of the fish. Feeding was performed until apparent satiation twice a day for 45 days. During the rearing period, the water quality was controlled at 9.15-29.5ºC, pH of 6.5-6.57, dissolved oxygen of 6.2-6.34 $mg.L^{-1}$, and ammonia of 0.19-0.36 $mg.L^{-1}$.

Parameters

Growth Performance

Feed Intake (FI)

Feed intake was calculated from the difference of initial diet weight and final diet weight in the container.

Weight Gain (WG) and Length Gain (LG)

Weight gain was calculated from the difference of final fish biomass weight (Wt) and initial fish biomass weight (Wo). Meanwhile, length gain was calculated from the difference of final fish biomass length (Lt) and initial fish biomass length (Lo).

Specific Growth Rate (SGR)

The SGR value ($\%.day^{-1}$) was calculated with the formula:

$SGR = \{[\ln(Wt) - \ln(Wo)] / t\} \times 100$ ……………………. (1)

whereas t is the total rearing period of polar blue cichlids.

Feed Conversion Ratio (FCR)

The FCR value was calculated with the formula:

$FCR = FI/[(Wt + Wd) - Wo]$ …………………….………. (2)

whereas Wd is dead fish weight.

Survival Rate (SR)

The SR value presents the percentage of fish populations at the final and initial rearing period.

Color Quality

Color Intensity Level

Color intensity was analyzed using the *Adobe Photoshop* software application by viewing the color libraries and the code for each color. The formula applied to obtain the color intensity level, based on the value of each color code: Color intensity level (total grid percentage,%) = (ΣGrid per color code/total number of Grids) × 100.

Total Chromatophores

Total chromatophores were counted manually with a microscope at 400× magnification at the end of the rearing period. Samples for total chromatophores counting in fish skin were prepared through a histological method with hematoxylin-eosin staining (Indriani et al., 2023).

Data Analysis

All data were analyzed using an Analysis of Variance (ANOVA) to determine a significant effect of the treatment on the growth performance and color quality of the fish. If there was a significantly different effect, the Duncan's multiple range test (DMRT) was applied to obtain the best treatment with optimal growth performance and color quality of the polar blue cichlids at 95% of confidence level.

RESULT AND DISCUSSION

Growth Performance

Table 1 presents the growth performance parameters data after fish were fed with various doses of *Spirulina* powder through dietary supplementation. The statistical analysis of all treatments indicates no significant difference *(p>0.05)* on the growth performance parameters, namely feed intake (FI), weight gain (WG), length gain (LG), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR).

Table 1. Growth Performance Over The Experimental Period (45 Days) of *Polar Blue Cichlid* Fed With The Four Experimental Diets

Parameters	Treatments			
	K	**A**	**B**	**C**
FI ($g.day^{-1}$)	0.88±0.10	0.88±0.19	0.74±0.19	0.89±0.03
WG (g)	3.34±0.30	3.18±0.11	2.82±0.34	3.34±0.24
LG (cm)	4.41±0.21	0.13±0.13	4.19±0.19	4.49±0.26
SGR ($\%.day^{-1}$)	0.85±0.27	1.00±0.27	0.79±0.46	0.82±0.06
FCR	2.69±0.87	2.30±0.05	3.41±2.06	2.59±0.20
SR (%)	82±7.70	71±16.78	44±34.21	69±3.85

Note: K = 0 g/kg diet, A = 10 g/kg diet, B = 20 g/kg diet , and C = 30 g/kg diet. Data are shown as mean±standard deviation; All data are insignificantly different at the 95% confidence level.

Although all data present no significant difference (p>0.05), several data indicate a similar condition in the previous studies. Rosid et al. (2019) reported that the dietary supplementation of *Spirulina* sp. powder showed no significant difference on the growth performance (WG, SGR, FCR, and SR) of goldfish at 1.5, 1.8, and 2.1 g/100 g diet. Tu et al. (2022) mentioned that the dietary supplementation of *Spirulina* powder at 3, 6, and 9 g/kg diet had no significant difference on growth performance (WG, FCR, SR, and SGR) of Discus fish (*Symphysodon* sp.). Erdogan (2019) showed that the dietary supplementation of *Spirulina* powder could produce no significant difference on growth performance (FI, WG, SGR, and FCR) of blue dolphin cichlids (*Cyrtocara moorii*) at 5, 10, 15 g/100 g diet. Therefore, the *S. platensis* powder supplementation in the diet has no alimentary effect on the growth performance of polar blue cichlids.

However, the FCR depicts a slightly higher value in this study than any previous studies in Oscar fish (*Astronotus ocellatus*) at 1.30-2.10 (Mohammadiazarm et al., 2021) and Nile tilapia (*Oreochromis niloticus*) at 1.35-1.80 (Al Mulhim et al., 2023), because the protein content in the diets was thought to be less than the protein required by fish to grow. For ornamental cichlids, the protein content in diets is commonly between 39-50% (Erdogan, 2019; Mohammadiazarm et al., 2021; Tu et al., 2022). Less protein content in diets than the protein requirement can cause a growth deprivation, as protein in the diet is utilized as the main growth component (Halver & Hardy, 2003). In addition, as polar blue cichlids are newly-cultured ornamental fish species in Indonesia, a proper diet for this fish is limited that urges the culturists to find appropriate diet for other fish species with similar taxonomy to polar blue cichlids.

Color Quality

As shown in Figure. 1, the dietary supplementation of *S. platensis* affects the color intensity of polar blue cichlids. Fish fed with spirulina-supplemented diet at 1-3 g/kg dose presents a more distinct color, specifically blue and black color, than the control treatment (0 g/kg). A distinct color in ornamental fish will be more attractive to the hobbyists, thus increasing the selling value of the fish. Therefore, spirulina powder can be applied to improve the color intensity of polar blue cichlids, before selling them to the market.

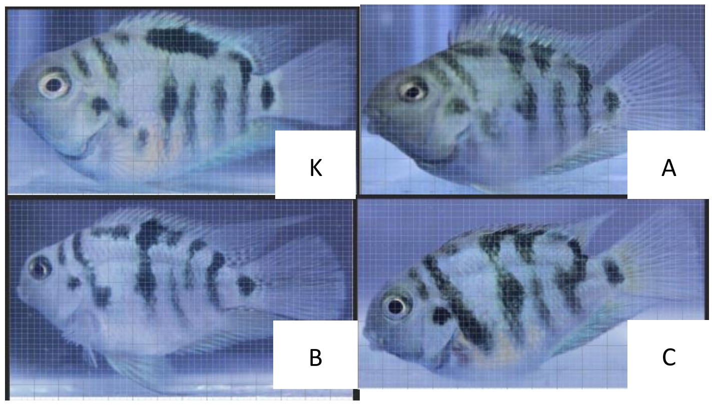

Figure 1. Color Intensity Of Polar Blue Cichlids After Feeding With Spirulina-Supplemented Diets.
Note: K = 0 g/kg diet, A = 10 g/kg diet, B = 20 g/kg diet , and C = 30 g/kg diet.

In addition to direct observation, color intensity in polar blue cichlids was also determined via *Adobe photoshop*, following the total grid percentage of blue color in the color library. The total grid percentage of blue color in each treatment is shown in Fig. 2.

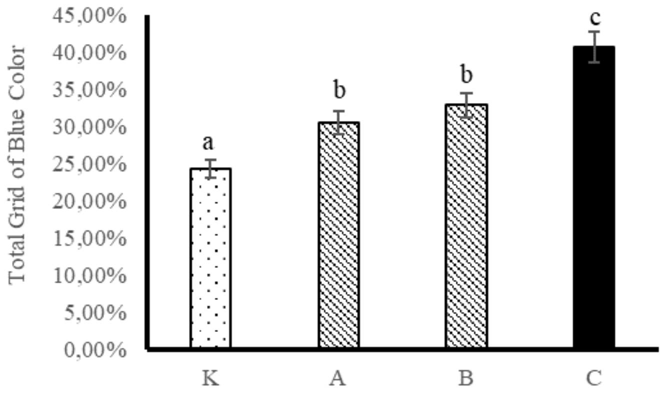

Figure 2. Total Grid Percentage of Blue Color In Polar Blue Cichlids After Feeding With Spirulina-Supplemented Diets.

Data are shown as mean±standard deviation. Different superscript letters show a significantly different value ($p<0.05$, DMRT 95%). K = 0 g/kg diet, A = 10 g/kg diet, B = 20 g/kg diet , and C = 30 g/kg diet.

The highest value of total grid percentage of blue color in polar blue cichlids was found in the C treatment (3 g/kg diet). A similar condition was also reported by Hadijah et al. (2020) in clownfish, that spirulina-supplemented diet with 30 g/kg dose could produce a higher color intensity level at 181.6. Meanwhile, Ren et al. (2021) mentioned that yellow river carp (*Cyprinus carpio*) fed with spirulina-supplemented diet at 0.8 g/kg dose produced a higher yellowness color and showed a better color intensity than without spirulina-supplemented diet (15.44 ± 1.41 vs 6.47 ± 0.86). Roohani et al. (2019) also described that Caspian brown trout (*Salmo trutta caspius*) presented higher lightness, redness, and yellowness color, when fish were fed with *Spirulina*-supplemented diets at 40, 60, and 80 g/kg dose. Therefore, these results confirm that *S. platensis* are rich in phycocyanin and carotenoids (β-carotene, canthaxanthin, astaxanthin, lutein, and zeaxanthin) as natural pigments, thus providing a better color performance in ornamental fish, including polar blue cichlids that have blue-colored body (Nakano & Wiegertjes, 2020; Alagawany et al., 2021; Bortolini et al., 2022; Edmond, 2022). Moreover, natural pigments are more environmentally-friendly without producing negative feedback to the fish, when supplemented to the diet. Natural pigments also assure other aspects for better performance in fish, namely health and resistance to various stressors (Gupta et al., 2007).

Furthermore, a higher total grid percentage of blue color in polar blue cichlids in C treatment is also proportional to the total chromatophores (Fig. 3). Meanwhile, other treatments showed no significant difference ($p>0.05$).

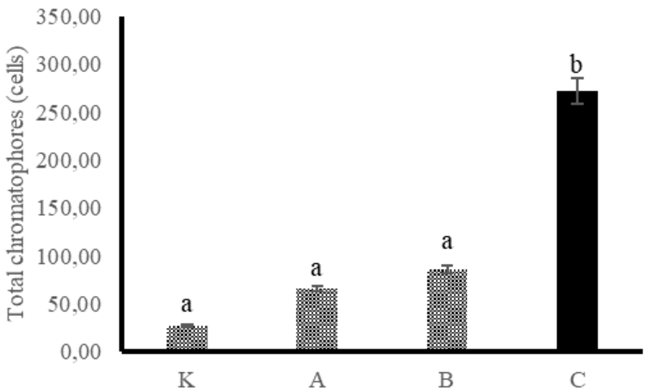

Figure 3. Total Chromatophores Of Polar Blue Cichlids After Feeding With Spirulina-Supplemented Diets

Data are shown as mean±standard deviation. Different superscript letters show a significantly different value ($p<0.05$, DMRT 95%). K = 0 g/kg diet, A = 10 g/kg diet, B = 20 g/kg diet , and C = 30 g/kg diet.

Natural pigments stored in the skin are utilized to optimize the performance of chromatophores. According to Luo et al. (2021), chromatophores are skin cells in the dermal layer that produce various pigments in fish. Chromatophores are unable to produce pigments on their own, thus requiring external pigments to optimize their performance in building the skin color (Gupta et al., 2007).

The higher total chromatophores indicate that the skin color of ornamental fish is optimal and evenly-distributed (Indriani et al., 2023). Similar results were also reported by Rahman et al. (2021) in goldfish, Saputra et al. (2023) in Betta fish (*Betta splendens*), and Satria et al. (2022) in rainbow boesemani fish (*Melanotaenia boesemani*) through *Spirulina* powder dietary supplementation at various dosage (6 g/kg diet and 1-1.5 g/kg diet, respectively). Moreover, a high number of chromatophores also depict that the natural pigments in *S. platensis* are absorbed perfectly and fish are in stable condition and resistant to stress. Barton (2002) stated that stress can cause several changes in behavior, morphology, and physiology of fish, including skin coloration. Moreover, Al Mulhim et al. (2023) reported that Nile tilapia fed with a *Spirulina*-supplemented diet with 50-100 g/kg diet obtained a higher resistance to stress condition, following a higher production of catalase and superoxide dismutase as stress-resistance enzymes. Therefore, it is suspected that the dietary supplementation of *S. platensis* powder can regulate the resistance level of the fish to stress conditions, thereby improving the color intensity and number of chromatophores in polar blue cichlids.

CONCLUSION

The results showed that the dietary supplementation of *S. platensis* powder had no significant effect on the growth performance (FI, WG, LG, SGR, FCR, and SR) of polar blue cichlids. However, the supplemented diets provide a better color intensity than without dietary supplementation, following a higher total grid percentage of blue color and total chromatophores in the fish. The best treatment that obtained the highest total grid percentage and total chromatophores were found in 30 g/kg diet dose of *S. platensis* powder. Therefore, 30 g/kg dose of *S. platensis* powder is effective to improve the color quality of polar blue cichlids, although providing no significant effect on the growth of the fish.

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