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Growth Response and Color Brightness of Betta Fish (*Betta splendens* (Regan, 1910)) Supplemented by Spirulina Powder from Algae *Arthrospira maxima* (Setchell and N. L. Gardner 1917))

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ABSTRACT

As a fighting fish and ornamental fish, the growth and color brightness of Betta fish (*Betta splendens*) are critical to be maintained in good conditions. Spirulina Powder (SP) from a blue-green microalga *Arthrospira maxima* is a nutrient source that can increase the performance of the fish. The research aimed to determine the effect of *A. maxima* in increasing brightness and growth in Betta fish. The experiment used a completely randomized design consisting of a control and three treatment groups with the addition of 5, 10, and 15% SP, respectively. The absolute weight, growth rate, and relative growth were assessed to evaluate the growth performance. In addition, the color brightness, blue color relative brightness, and blue chanel ratio were evaluated using image processing software. The results indicated that adding 15% SP significantly increased the growth and color brightness of Beta fish. Meanwhile, the blue-color change in fish is slightly different among all treatments. Based on this research, SP that contained carotenoids is a potential source for enhancing the color brightness of Betta fish.

Keywords: Blue-green microalga, Fish feeds, Siamese fighting fish.

INTRODUCTION

Siamese fighting fish or Betta fish (*Betta splendens*) were used as fighting fish and ornamental fish. Therefore, these fish become the object of the contest to be competed in terms of combat and aesthetics because of the unique patterns in body colors and the tail color that hangs wide (Saekhow *et al*., 2018). Its uniqueness has made the Betta fish have good prospects in the market (Rachmawati, 2016; Cathurani and Rajapakshe, 2020).

Pigments of Betta fish on the body's surface are mainly red, green, yellow, and blue. The expression of these colors are regulated by the genes for melanin, iridescence pigments and pigment density (Monvises *et al*., 2009). The colors on pigment cells or chromatophores and can change by environmental condition, habitat and the external stimuli. In addition, fish absorb pigments through diet, because the colors cannot produce endogenously (Sefc *et al*., 2014). Therefore, the breeders or fish farmers have been trying to control the color appearance by using an external color enhancer that is added to the feed.

Microalgae with high protein content are alternative and prospective fish feeds

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(Liang, 2015; Li *et al*., 2015). As a photosynthetic organism, microalga synthesizes pigments that can be transferred to fish through their diet (Mejia-Mejia *et al*., 2021). Microalgae from *Arthrospira* genera, or Spirulina as a trading name, have the potential as a source of antioxidants and high protein (Miranda *et al*., 1998). *Arthrospira* contains phycocyanin and other carotenoids as a group of natural color pigments that are expected to brighten and increase the color of Betta fish after being mixed with feed (Romay *et al*., 2003).

The quality of color brightness and the growth of Betta fish are crucial. Therefore, both parameters need to be improved and maintained. This experiment aimed to determine the effect of the addition of SP to Betta fish feed with a specific concentration. It is expected that the treatment will increase the brightness and the growth of Betta fish.

MATERIALS AND METHODS

Materials

This research was conducted using samples of Betta fish aged $^{+}2$ months whose color had not yet appeared to evaluate changes in brightness and growth. The fish was collected from the broodstock of the blue variant selected by local traders. Meanwhile, SP derived from the dry biomass of *Arthrospira maxima* was obtained from the Applied Microalgae Laboratory of the National Research and Innovation Agency (BRIN). The nutritional composition of the powders mentioned above can be seen in Table 1. In addition, a private laboratory analyzed this nutritional profile.

The equipment used in this study were aquariums (15×10×15 cm^3), a bucket, a Canon EOS 3000D camera, a hose, a drain, an RGB (ImageJ) color measurement application, an RGB to HSB conversion application, a Philips HR2116 blender, MO-20 MITO oven, 1 L plastic for feed manufacturing, Taffware Digipounds digital scales, filters, black cloth, gauze, and Laptop.

Fish Feed Preparation

The composition of fish feed refers to the study of Sholichin *et al.* (2012) and are reported in Table 2. The treatment in this experiment was divided into four groups as follows:

1. Control: The addition of 0% SP
2. Treatment A: The addition of 5% SP
3. Treatment B: Addition of 10% SP
4. Treatment C: The addition of 15% SP

The SP was added into the Betta fish feed that contained soybean meal, fishmeal, fine bran, tapioca flour, vitamin mix, minerals, and fish oil. All compositions were mixed until they reached 100% according to the formulation of 30% protein per 100 grams calculated using the Pearson square method.

Experimental Design

The experiment was performed using a

Table 1. Nutrients composition of Spirulina powder from *Arthrosphira maxima* dried biomass used in this experiment.

Parameter	Unit	Result	Method
Total energy	kCal 100 g^{-1}	341.32	Calculation
Water content	%	9.98	Gravimetri
Ash content	%	11.34	Gravimetri
Lipid	%	5.32	Soxlet
Protein	%	46.99	Kjeldhal
Carbohydrate	%	26.37	by different
Vitamin A	μg 100 g^{-1}	435.00	HPLC
Vitamin E	μg 100 g^{-1}	0.20	HPLC

Completely Randomized Design (CRD) with five replicates. All variables were homogeneous and treated as a single unit so that no other factors were considered influential for the results.

Initially, the fish were acclimatized for four days and fed by the commercial feed without a mixture of spirulina powder. Fish were fed two times daily, in the morning at 09.00 WIB and in the afternoon at 17.00 WIB, for 20 days. The growth and brightness parameters were compared between the initial day (day-1) and the end of treatment (day-21).

Data Analysis

The weight of each fish was measured gravimetrically to calculate the change in mass. The following formula calculated the Growth (G):

$$G = Wt - W0$$

Meanwhile, the Growth Rate (GR) and Relative Growth (RG) were calculated using the following equation:

$$GR = (Wt - W0) / t$$

$$RG = [(Wt - W0) / W0] \text{ x } 100\%$$

Where, G= Growth (g); GR= Growth Rate; RG= Relative Growth; Wt= Weight of fish at the end of rearing (g); W_0 = Initial fish Weight (g), t= Duration of feeding periods (20 days)

The initial color of Betta fish was measured before treatment, then, photographs were taken at the end of the experiment of each fish sample in the aquarium. Black background to counterbalance the light fluctuations was set up while taking photos. The distance of the camera lens was about 10-15 cm from the aquarium. Therefore, each Betta fish sample was shot with the same light, background, and space. The colors obtained from the image samples were analyzed using ImageJ software, a robust tool for processing the image (Broeke *et al*., 2015). The parameters obtained from the software include the mean, number, and frequency of color in the form of a histogram. The histogram of blue Chanel was selected to analyze the change in blue paint. The external software RGB to HSB Calculator was used for converting RGB to HSB (Hue, Saturation, Brightness) model.

The color intensity was evaluated based on the change of blue color. It was determined to know the effect of natural blue-green color derived from phycocyanin. As a blue-green algae group, the dried biomass of *Arthrospira maxima* contains phycocyanin. Therefore, the relative blue color was calculated from the ratio of blue to the total value of RGB using the following equation (Richardson *et al*., 2007):

$$RBB = B / (R + G + B)$$

Where, RBB= Relative Blue color Brightness; B= Blue color value detected from image; R= Red color value detected from image, G= Green color value detected from image.

The mean of the histogram was selected only from the blue-color channel that was determined manually based on ImageJ

Table 2. Fish feed composition used in this experiment.

No	Ingredients	Percentage (%)			
		Control	A	B	C
1	Spirulina Powder (SP)	0	5	10	15
2	Soybean flour	29	27.3	25.6	24
3	Fish meal	38	36.3	34.6	33
4	Fine bran	27	25.3	23.6	22
5	Vitamin mix	1	1	1	1
6	Mineral	1	1	1	1
7	Fish oil	4	4	4	4

calculation after the background of the image was cleared. This technique was proper to compare the shift of color before and after treatment. The blue-color change was obtained from the histogram-mean ratio between day-21 and day-1. An example of the histogram from the blue-color channel is presented in Figure 1.

IBM SPSS Statistics 26.0 was used to determine the mean, deviation standard, ANOVA, and Duncan test. The clustered combo and regression graphs were created using Excel from Microsoft Office Word software. The linear regression equation obtained the coefficient of determination (R^2) and correlation coefficient (r). According to Chin (1998), R^2e was categorized as strong impact if $R^2 > 0.67$; moderate if $R^2 = 0.33–0.67$; weak if $0.19 < R^2 < 0.33$.

Environmental Parameters Monitoring

Environmental parameters such as water temperature and pH were measured daily using a thermometer and a pH meter, respectively, while dissolved oxygen was measured using a DO-meter. The water in each aquarium was changed every five days during the experimental period.

RESULTS

SP used in this study contained 46.99% protein, 26.37% carbohydrate, and 5.32% lipid in each dried weight of biomass (Table 1). Besides, the source of protein for Betta fish was derived from soybean and fish meal (Table 2).

The impact of SP on the brightness and growth of fish samples is shown in Figure 2. The saturation and growth of Betta fish were increased either treated with SP or without adding it into the feed. The addition 5%, 10%, and 15% of SP apparently elevated the brightness higher than the control 1.43; 3.10; 4.48 times, respectively. The rate of brightness was higher than growth along with the increase in SP concentration. Based on Figure 2, the addition of SP increased the absolute weight better than the control.

The linear relationship and correlation

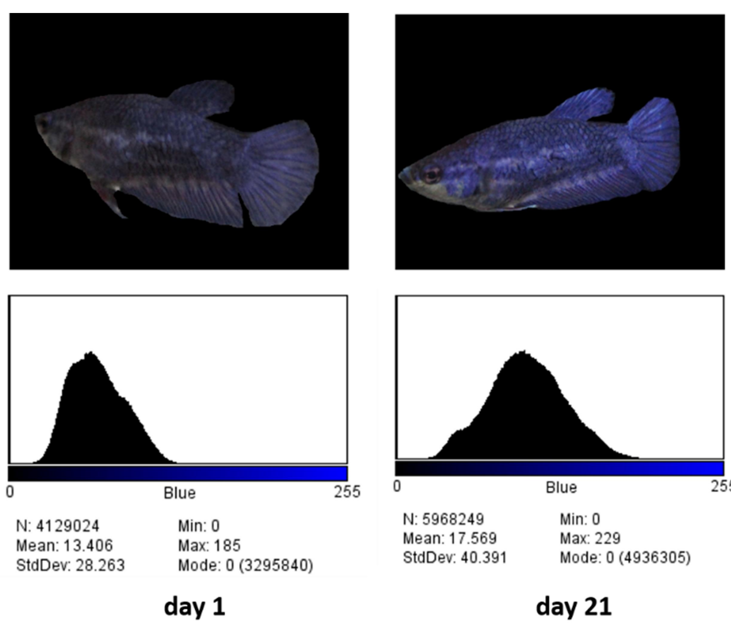

Figure 1. Blue color-change measurement using the ratio of histogram-mean between day-1 and day-21. The vertical axis of histogram indicated the frequency of appearance of blue color (pixel) in certain hue.

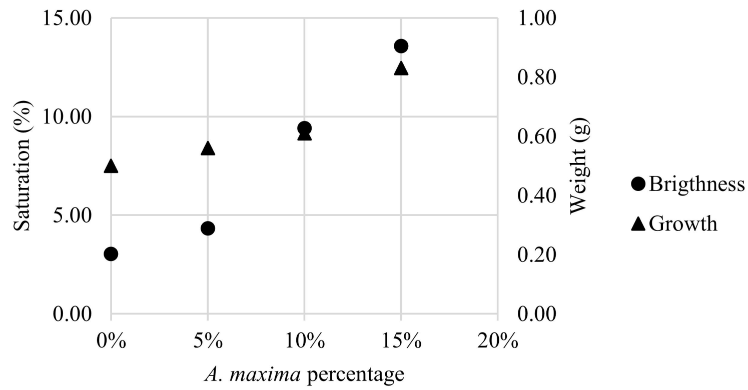

Figure 2. Effect of SP in daily experimental diet on brightness and growth of Betta fish.

between growth and brightness parameter were determined (Figure 3). Growth and brightness are strongly correlated to each other with correlation coefficient (r) of 0.95. In this study, the growth gives 89.66% positive impact to brightness while the rest of 10.34% is influenced by other factors.

The growth of Betta fish increased along with the concentration of SP added into the feed. The addition of 15% SP into the feed increased the weight up to 0.83 gram or 1.66 times heavier than the weight of fish that were not fed with Spirulina-containing feed. In addition, 15% SP resulted in significantly different growth (α= 0.05) compared to other treatments. In general, the fish growth rate increased in the fish fed with SP. However, it showed no statistically significant difference among the four treatments (Table 3).

The brightness change on Betta fish varied after 20 days of treatment. The brightness based on the RGB (Red, Blue, Green) after converted into HSB model was significantly increased along with increased SP concentration added into the feed (Table 3). The shift of color for each Betta fish group is represented in Figure 4.

Beside the brightness of the full color, the blue color change was evaluated. The result showed that either based on relative blue color brightness value or the ratio of histogram-mean on the blue channel is not significantly different from that of the

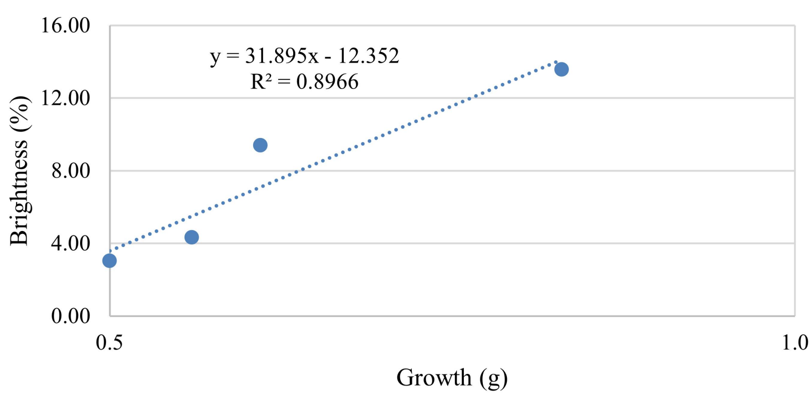

Figure 3. Relationship between brightness and growth of Betta fish (*Betta splendens*) in this experiment.

Table 3. Summary of Betta fish growth as impact of SP addition into the fish feed.

Group	Initial weight (g)	Final weight (g)	Growth (g)	Growth rate (g d^{-1})	Relative growth (%)
Control	0.41 ± 0.18	0.91 ± 0.09	0.50 ± 0.10 [a]	0.02 ± 0.005 [a]	120.16
A	0.35 ± 0.07	0.91 ± 0.10	0.56 ± 0.10 [a]	0.03 ± 0.005 [a]	158.49
B	0.37 ± 0.06	0.95 ± 0.14	0.61 ± 0.14 [a]	0.03 ± 0.007 [a]	171.03
C	0.30 ± 0.10	1.13 ± 0.06	0.83 ± 0.06 [b]	0.04 ± 0.003 [a]	277.78

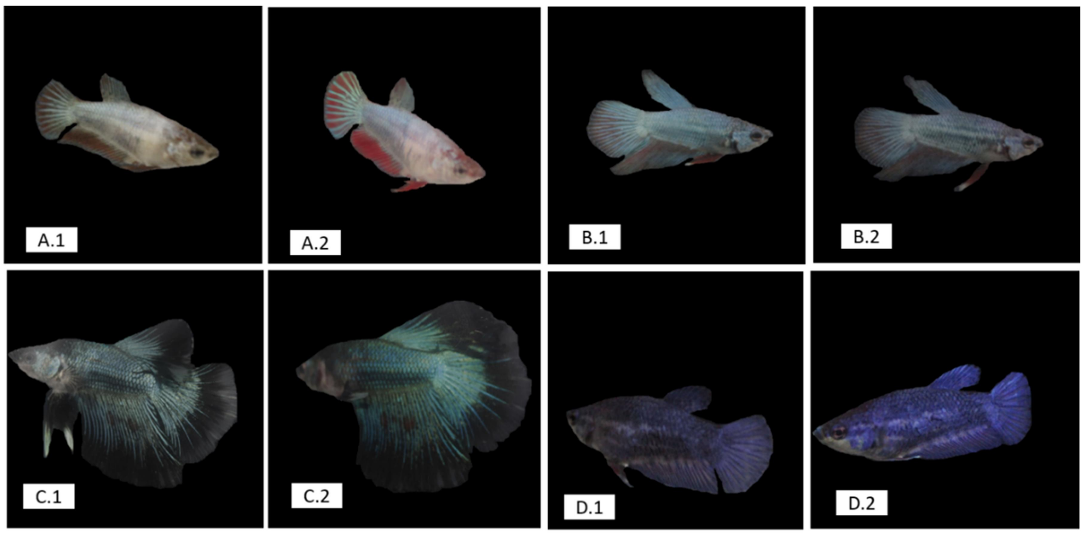

Figure 4. Visualization of color-change on representative fish before (A1, B1, C1, D1) and after (A2, B2, C2, D2) applying *A. maxima* into the diet. A indicated treatment feed without containing SP, while B, C, and D showed treatment feed containing 5, 10, and 15% SP, respectively.

control (α= 0.05). However, the quantity of blue color is slightly higher for the 15% SP treatment.

The 15% treatment occupied the highest brightness, followed by the 10% treatment and 5% treatment, while the lowest was found in the control (without adding SP) (Table 4). In addition, 15% treatment enhanced the quantity of the blue color of the Betta fish slightly higher than others. However, based on the Duncan test, the increase of blue color was not significantly different among treatments (α= 0.05).

The environmental condition among groups were in the normal range (Table 5), the environmental parameters during the experiment were still in the tolerable range for Betta fish life and the process of increased pigmentation. The optimal temperature of ornamental fish such as Betta fish is in the field of 25-28°C, and a suitable water pH is in the slightly acidic to a neutral range of 6.5-7.5

DISCUSSION

The increase of weight in Betta fish because of SP nutrient composition follows what Chu *et al.* (2010) stated. SP from *Arthrospira (Spirulina) maxima* has high protein (Souza *et al.*, 2021), fatty acid, vitamin, and antioxidant components, which are used by *B. splendens* for growth.

The results of ANOVA analysis found that the growth of Betta fish fed by 15% SP was significantly different from other treatments ($P < 0.05$). The 15% treatment resulted in the higher absolute weight (growth) and

Table 4. Summary of brightness and color-change as impact of SP addition into the fish feed.

Group	Color brightness	Blue color relative-brightness ratio	Blue channel ratio
control	3.03 ± 0.49 [a]	1.06 ± 0.05 [a]	1.09 ± 0.27 [a]
A	4.33 ± 1.46 [a]	1.06 ± 0.09 [a]	0.98 ± 0.15 [a]
B	9.4 ± 1.31 [b]	1.06 ± 0.04 [a]	1.18 ± 0.42 [a]
C	13.57 ± 2.30 [c]	1.13 ± 0.06 [a]	1.39 ± 0.63 [a]

Table 5. Environmental parameter after treatment.

Group	Temperature (°C)	Water quality (DO) (mg L^{-1})	pH
control	27.6	3.0-3.9	6.5
A	27.4	3.5-4.2	7
B	26.8	3.2-4.0	6.5
C	27.1	3.3-3.7	6.5

statistically different compared to other treatments, so, this percentage is appropriate for different feed formulations. While compared with research conducted by Asrami *et al*. (2019) with the administration of Spirulina in fish, *Poecilia reticulata* increased in mass reached 123.95%, so that *A. maxima* obtained a higher percentage of 277.78%.

In several studies, SP has been widely used as an additive to increase the color of the fish *Xiphophorus helleri* (Vasudevan *et al*., 2006), *Poecilia reticulata* (Asrami *et al*., 2019), *Maylandia Lombardoi* (Karadal *et a*l., 2017). *Arthrospira platensis* and *A.maxima* are the most important species in the genera of *Arthrospira* that are widely used for many applications (Habib and Parvin, 2008). Microalgae *Arthrospira platensis* (Hadijah *et al*., 2020), *Haematococcus Pluvialis* (Chapman and Miles, 2018), and *Porphyridium cruentum* (Syaifudin *et al*., 2016) are reported to be used as color brightness enhancer of fish. However, no research report has been found on the addition of SP from *A. maxima* to Betta fish feed. This study reported that adding SP from *A. maxima* up to 15% may increase the color brightness, indicated that SP contains a color enhancer compound. However, an increase in concentration is required to elevate the blue color quantity significantly. Therefore, further experiment with SP concentration of more than 15% needs to be conducted to reveal the impact of phycocyanin on the blue color change.

A combination of chromatophores effects mainly generates the blue coloration in Betta fish. Based on the ultrastructural study, a manifestation of blue colors in fish was influenced predominantly by the composition of iridophores and melanophores as a dermal pigmentary unit (Amiri and Shaheen, 2012). Phycocyanin from Spirulina or additional color sources incorporated into feed can affect the color of *B. splendens* after being digested in the intestine, then entering into the lymphatic channel. After that, fine particles will be transferred to the liver through the blood circulation system. Arriving in the liver, binding proteins synthesized by the liver will retain these fine particles and flow to tissues using carrier protein. Therefore, the pigments will be absorbed in the cells and stimulate the increase of chromatophores. Finally, the cells express pigments in the dermis of fish skin.

Internal and external factors can influence the increase of brightness in fish. Internal factors are age, size, sex, genetics, and absorption of nutrients in the feed, while the external factors are water quality, lighting, and the content of the fish feed (Indarti *et al.* 2013). In this study, these factors were homogenized as controlled variables, except the SP percentages applied into fish feed that was distinguished as the independent variable. This study reports that the growth strongly affected the color brightness of fish samples (Figure 2).

Carotenoids are used as compounds for a color-brightness enhancer in much research. Spirulina contains carotenoids such as phycocyanin, carotenes, chlorophyll-a, and astaxanthin (Vonshak, 1997; Prabhath *et al*., 2019; Yustiati *et al*., 2020; Margareta *et al*., 2021). These compounds act as an antioxidant that is important for fish health and, indirectly, for growth. Betta fish use these compounds to maintain their color and immune response (Pleeging and Moons, 2017). Based on this study, 15% SP was a more effective treatment to increase growth, brightness and slightly increase the quantity of blue color compared to the treatment without the addition of SP. Carotenoids were also contained in soybean, so, the color changes were recognized too in the control group, even though they were lower than in other treatments. This observation follows Phonna *et al*. (2022), who revealed the impact of carotenoids to increase the growth and color brightness of clownfish (*Amphiprion ocellaris*) and comet fish (*Carassius auratus*), respectively.

In conclusion, SP from algae *Arthrospira maxima* have the potential as an additional fish feed to increase the brightness and growth of *B. splendens*. Based on this study, the addition of 15% SP to fish feed significantly increases the brightness and growth compared to other treatments. However, the effect of SP on blue color quantity is not entirely different from others in this concentration. Therefore, further research to find the best SP percentase for the blue color enhancement in Betta fish is necessary.

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واکنش رشد و درخشندگی رنگ ماهی بتا ((*Betta splendens* (Regan, 1910) به مکمل غذایی پودر اسپیرولینا از جلبک *Arthrospira maxima* (Setchell and N. L. Gardner 1917)

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چکیده

برای بقای ماهی بتا (Betta splendens) به عنوان یک ماهی جنگجو و زینتی، حفظ رشد و روشنایی رنگ در شرایط خوب ضروری است. پودر اسپیرولینا (SP) از ریزجلبک سبز آبی Arthrospira maxima یک منبع مغذی است که می تواند عملکرد ماهی را افزایش دهد. این پژوهش با هدف تعیین تأثیر *A. maxima* در افزایش درخشندگی و رشد ماهی بتا انجام شد. دراین آزمایش از یک طرح کاملا تصادفی شامل یک گروه شاهد و سه گروه تیماری با افزودن ۵%، ۱۰% و ۱۵% SP استفاده شد. برای ارزیابی رشد، وزن مطلق، سرعت رشد و رشد نسبی مورد ارزیابی قرار گرفت. علاوه بر این، درخشندگی رنگ، درخشندگی نسبی رنگ آبی و نسبت کانال آبی (blue chanel ratio) با استفاده از نرم افزار پردازش تصویر ارزیابی شد. نتایج نشان داد که افزودن SP%۱۵ به طور قابل توجهی رشد و درخشندگی رنگ ماهی بتا را افزایش داد. همزمان، تغییر رنگ آبی

در ماهی در بین تمام تیمارها کمی متفاوت است. بر پایه این پژوهش، SP حاوی کاروتنوئیدها منبع بالقوه ای برای افزایش درخشندگی رنگ ماهی بتا است.