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Spirulina plantesis flour supplementation to increase the growth and brightness of the color of Betta fish (*Betta splendens*) Regan, 1910

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Abstract. The cultivation of ornamental fish has not yet reached an adequate level to consistently produce high color quality, which is a key determinant of the fish's market value. This study aims to investigate the Effect of *Spirulina plantesis*, which is believed to contain carotenoids that can enhance the color of freshwater ornamental fish, specifically *Betta splendens*. A quantitative approach using a Completely Randomized Design (CRD) experimental model was employed to test the hypothesis, with the results analyzed statistically and described in detail. The study found that treatment 2, involving a 1% concentration of *Spirulina* powder in artificial feed, resulted in the best increase in color intensity, as indicated by a more yellow and darker hue compared to the control, P3, and P4 treatments. The study suggests that future research should include a larger sample size to achieve optimal results.

1 Introduction

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Betta fish are among the most popular freshwater ornamental fish, attracting enthusiasts from various age groups, including children, teenagers, and adults. Their high popularity is due to their unique color patterns, which are a significant attraction [2]. The vibrant colors of betta fish generate high interest among ornamental fish enthusiasts. In addition to their striking colors, various tail types and shapes also contribute to their appeal. Some common tail types include the 180-degree or half-moon tail known as "half-moon," the "crown tail" with a crown-like tail, the rounded tail, and the short tail, often referred to by enthusiasts as "plakat" [3].

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 Hany Handajani

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Abstract. The cultivation of ornamental fish has not yet reached an adequate level to consistently produce high color quality, which is a key determinant of the fish's market value. This study aims to investigate the Effect of *Spirulina platensis*, which is believed to contain carotenoids that can enhance the color of freshwater ornamental fish, specifically *Betta splendens*. A quantitative approach using a Completely Randomized Design (CRD) experimental model was employed to test the hypothesis, with the results analyzed statistically and described in detail. The study found that treatment 2, involving a 1% concentration of *Spirulina* powder in artificial feed, resulted in the best increase in color intensity, as indicated by a more yellow and darker hue compared to the control, P3, and P4 treatments. The study suggests that future research should include a larger sample size to achieve optimal results.

1 Introduction

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Betta fish are among the most popular freshwater ornamental fish, attracting enthusiasts from various age groups, including children, teenagers, and adults. Their high popularity is due to their unique color patterns, which are a significant attraction [2]. The vibrant colors of betta fish generate high interest among ornamental fish enthusiasts. In addition to their striking colors, various tail types and shapes also contribute to their appeal. Some common tail types include the 180-degree or half-moon tail known as "half-moon," the "crown tail" with a crown-like tail, the rounded tail, and the short tail, often referred to by enthusiasts as "plakat" [3].

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However, despite the high market demand, betta fish farming still faces challenges, particularly in color quality. Low color quality can lead to unstable market prices and even depreciation. Success in the ornamental fish trade largely depends on the brightness and quality of the fish's color. Thus, enhancing color quality in fish farming is a primary solution to increase consumer interest, economic value for farmers, and conservation of fish biodiversity [4].

The vibrant colors in fish are only prominent during specific periods, particularly during the breeding season. Betta fish, being territorial, are ideal as ornamental fish due to their diverse and beautiful colors. These colors range from primary hues like green, blue, and yellow to combinations of other colors such as copper, blue metallic, multicolor, and various other variations [5].

Several factors influence the color intensity of betta fish, including internal and external factors. Internal factors are inherent or genetic traits within the fish, which are fixed. On the other hand, external factors include water quality, light, environmental conditions, and a diet containing high nutrition and carotenoid sources [6]. Fish can only synthesize black and white pigments, while other colors like red, orange, and yellow cannot be directly synthesized without providing carotenoids in their diet. Therefore, color formation in ornamental fish heavily depends on the carotenoid content in their feed [7].

Carotenoids are naturally found in the fish's natural habitat and in some algae species, one of which is *Spirulina*, a blue-green microalga with a spiral shape and many species. However, only three species have been extensively studied due to their high nutritional value, one of which is *Spirulina platensis*. *Spirulina sp.* is widespread and serves as a high-nutrient feed for shrimp and fish larvae. It contains vitamins, minerals, and antioxidants (carotenoids and phycocyanin) and is often used in food and medicine. *Spirulina sp.* accumulates up to 1.0% carotenoids in its dry Weight, making it a viable natural source to enhance the color pigments in betta fish [8].

Carotenoids are organic pigments in the form of lipophilic isoprenoid molecules, consisting of carotenes like beta-carotene, alpha-carotene, and lycopene, and xanthophylls like lutein, astaxanthin, zeaxanthin, violaxanthin, laraxanthin, and fucoxanthin [9]. Carotenoids are found in chloroplasts and chromoplasts in plants and organisms such as algae, photosynthetic bacteria, non-photosynthetic bacteria, and non-photosynthetic fungi. The reddish-yellow pigment from beta-carotene is found in several algae species, including *Dunaliella salina*, *Haematococcus pluvialis*, *Chlorella*, and *Spirulina platensis* [10].

Previous studies, such as those by Nafisah [11] indicate that one of the carotenoids in *Spirulina* that contributes to color brightness in fish is astaxanthin, which can be used to optimize the environment for enhancing fish color phenotypes. Astaxanthin, a type of xanthophyll, has a higher antioxidant activity than other carotenoids. Additionally, it is the main carotenoid in aquatic organisms like shrimp, fish, and lobsters, with essential biological functions such as pigmentation, UV protection, essential fatty acids, and a significant impact on immune responses in several animal studies [12].

Aquatic animals cannot synthesize carotenoids in their bodies, so fish must obtain carotenoids through their diet, which can be supplemented with feed additives to improve and enhance their color quality [9]. Carotenoids can come from chemical sources but are not recommended due to safety concerns for both the fish and the aquatic environment. Ideally, carotenoids should come from natural sources like plants. However, excessive or improper dosing of natural pigments in feed may not improve color quality and could even reduce the color value [13]. Some research found that feeding effectively enhanced the color of the red swordtail fish, *Xiphophorus helleri* [14]. Further, a relevant study by Gumilara [15] showed that adding *Spirulina sp.* powder to goldfish feed did not affect the fish's survival rate. However, one of the treatments with 6% *Spirulina sp.* per 100g of artificial feed resulted in the highest color intensity.

Based on the issues surrounding fish farming and the need to improve color quality, this study aims to provide valuable information to the community, encouraging ornamental fish farming as an alternative profession with a high market value through color quality enhancement. Therefore, research is needed to determine the precise dosage of color pigments added to feed. The addition of *Spirulina sp.* powder to artificial feed for betta fish is proposed to test its impact on color enhancement.

2 Materials and Methods

The subjects of this study were 12 *Betta splendens* (Betta fish), specifically of the Plakat type, all from the same broodstock and aged 3 months. Before the experiment, the fish underwent a 2-day adaptation period and were then placed in individual test media, with one fish per aquarium or test media.

2.1 Research Method

This study employed a Completely Randomized Design (CRD) experimental model with four treatments and three replications. The treatments involved the addition of *Spirulina sp.* powder to artificial feed at different concentrations: 1% (1g/100g of feed), 3% (3g/100g of feed), 5% (5g/100g of feed), and a control with no *Spirulina sp.* added.

The treatments were as follows:

P1: No *Spirulina sp.* powder added (control)

P2: Addition of *Spirulina sp.* powder at 1g/100g of artificial feed

P3: Addition of *Spirulina sp.* powder at 3g/100g of artificial feed

P4: Addition of *Spirulina sp.* powder at 5g/100g of artificial feed

The Completely Randomized Design (CRD) in this study followed a linear model based:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij} \quad (1)$$

Where:

Y_{ij} = Observed data for treatment i and repetition j

μ = Overall mean or central value of the population

τ_i = Effect of treatment i

ε_{ij} = Experimental error for treatment i and repetition j

2.1.1. Observations

The primary parameters observed during the maintenance period included the intensity and quality of the fish's color, growth in length and Weight, and survival rate. The color observations focused on the body of the fish. Color intensity in the test fish, *Betta splendens*, was observed every ten days over a 30-day period. The observations were conducted quantitatively by comparing the fish's color against the Toca Colour Finder (TCF) standard. As modified for this study, the TCF color measurement tool is shown in Figure 1.

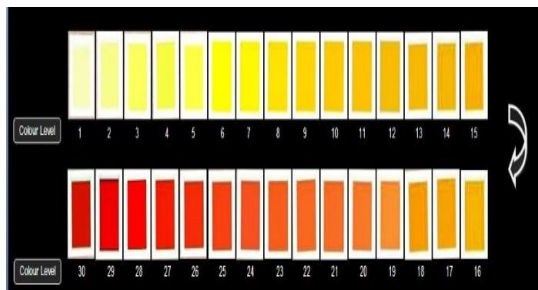


Fig. 1. Toca color finder

At the end of the study, the color of the test fish will be measured using a Colour Reader of type CR-10. The color differences will be calculated by determining the Delta E values for the treatments with *Spirulina* sp. concentrations of 1%, 3%, and 5% compared to the control (standard). The formula used for this calculation follows the LAB color model from the Commission International de l'Eclairage (CIE), specifically CIELAB 1976, with the Hunter method as described by Rulaningtyas [17].

Delta E (ΔE) is used to quantify the color difference between the sample and the standard. It is calculated using L, A, and B values obtained from the Colour Reader. If ΔE falls outside the tolerance range, the L, A, and B parameters also fall outside the tolerance range. Additionally, ΔC (chroma difference) and ΔH^* (hue angle difference) are other related metrics in the CIELAB scale [18], but this study will focus on ΔE using the following formula:

$$\Delta E = \sqrt{L^2 + A^2 + B^2} \quad (2)$$

Absolute Weight Gain will be calculated using the formula by Weatherley (1972) as adapted by [11]

$$W = W_t - W_0 \quad (3)$$

W: Pertambahan bobot mutlak ikan (g).

Wt: Bobot ikan pada waktu ke-t (g).

W0: Bobot ikan pada waktu ke-0 (g).

$$W = W_t - W_0 \quad (4)$$

Where:

W = Absolute weight gain of the fish (g)

Wt = Weight of the fish at time t (g)

W0 = Weight of the fish at time 0 (g)

Absolute Length Gain is the difference between the length of the fish from the head to the tail at the start and end of the study. It will be calculated using the formula:

$$P_m = L_t - L_o \quad (5)$$

Where:

Pm = Absolute length growth (cm)

Lt = Average length at the end of the study (cm)

Lo = Average length at the start of the study (cm)

The next step was to observe the water quality. The water quality parameters monitored during the study included temperature measured with a thermometer, pH assessed with litmus paper, and dissolved oxygen (DO) measured using a DO meter. Water quality measurements were taken three times during the maintenance period: once during the first ten days of the study, once in the middle, and once at the end. The results of the water quality observations were then compared with the standard water quality guidelines for betta fish habitat.

2.2 Broodstock Management

Broodstock management involved the selection of broodstock that were ready to spawn, had mature gonads, and were of sufficient age—females over two years and males over one year. The selected broodstock had a perfect, non-deformed body shape, sharp and non-graduated body color, and a well-defined pattern from head to tail without blockiness. Before spawning, the broodstock was maintained by feeding them to support growth and maintain color. Feed was provided 2-3 times daily, in the morning and evening, with 200 grams per feeding. The commercial feed was fermented with probiotics and vitamins to ensure easy digestion. Broodstock was transferred from the spawning pond in the evening. The transfer process began by netting the broodstock, which were then placed into plastic bags filled with water and supplemented with oxygen. Different types of broodstock could be packed together, but males and females were kept separate. However, female broodstock containing eggs could be packed with males if the distance between the broodstock pond and the spawning pond was short. Transfers were conducted in the evening when the temperature was lower, reducing stress on the fish. After spawning, the koi broodstock was quarantined and then placed into a broodstock pond to prevent the spread of pathogens.

3 Results and Discussion

3.1 Color Change

In the color intensity testing of Betta fish, a modified Toca Colour Finder (TCF) was used, featuring 30 different color change numbers, each showing a 20% contrast. Testing with TCF was conducted weekly over the 30-day study period, resulting in four tests: at the first week, second week, third week, and fourth week of the study.

The betta fish used in the study as test subjects were of the same age and species. According to the table above, there was no observable color change in the fish after being fed the artificial feed without the addition of *Spirulina* sp. powder (control), as well as in treatments P2, P3, and P4, which included *Spirulina* sp. powder in the artificial feed at the specified concentrations. Therefore, it can be concluded that the first week did not impact the betta fish, regardless of whether *Spirulina* sp. powder was added to the artificial feed.

Based on the results of the TCF testing described above, the test subjects in the P1 (control) group showed a color improvement, though not significant, with a value increase from 18 in week 1 to 19 in week 2. In contrast, P2, which involved adding 1% *Spirulina* sp. to the artificial feed, resulted in the highest color improvement among all treatments, with a value increase of 1.6, from 18 in week 1 to 19.6 in week 2. For P3, with a 3% *Spirulina* sp. addition to the feed, the test subjects showed a color improvement of 1.2, increasing from 18 in week 1 to 19.3 in week 2. On the other hand, P4, with a 5% *Spirulina* sp. addition, demonstrated a color improvement similar to that of the control group, with a final value of 19, indicating an increase of 1 compared to week 1. Based on these results, treatment P2 resulted in the highest color improvement compared to the other treatments, followed by P3 with a difference of 0.4, and finally, P4 and the control group had similar color improvement results.

Based on the table above, the TCF test results for week 3 indicate no color improvement in treatment 1 (control) and treatment 4 with a 5% *Spirulina* sp. addition from 100g of artificial feed. The color values for these treatments remained the same as in week 2. In contrast, treatment 2 showed a color improvement of 1.4, with the color value increasing from 19.6 in week 2 to 21 in week 3, based on the average color value. Treatment 3, with a 3% *Spirulina* sp. addition, experienced a color improvement of 0.3, with the average color value rising to 19.33 compared to week 2. Therefore, the results suggest that color

improvement in week three was observed in treatments 2 and 3, with treatment 2 showing a higher increase of 1.4 compared to treatment 3.

On week 4, the final week of the study, color testing of the test animals was conducted using the TCF, as shown in the table above. The results indicate that treatment 1 (control) did not experience any color improvement compared to previous weeks. In treatment 2, there was a color improvement with an average increase of 0.6 from week 3, resulting in a color value 19.6. Both treatments 3 and 4 showed a color increase of 0.3, with treatment 3 reaching a color value of 20.3 and treatment 4 reaching 19.3. Based on these results, treatment 1 (control) did not show any changes, while treatment 2 experienced a higher increase, although not as much as in the previous week. Treatments 3 and 4 had the same, non-significant increase of 0.3 compared to week 3. After analyzing the TCF results for each week, a descriptive statistical table will be created in graphical form, combining the mean values obtained from each treatment across all replicates, as shown in the following table.

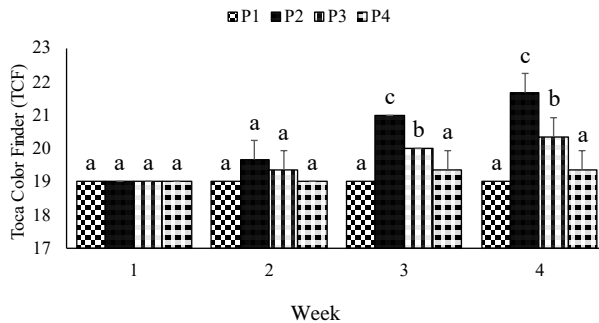


Fig 2. Weekly color changes in the fish's body using the Toca color finder score. Test statistical scores every week using Anova. Different notations indicate significant differences in the TCF score for each treatment.

The bar graph on the left represents the results for Treatment 1 (P1), or the control group, with feed containing no *Spirulina* sp. The following bar shows Treatment 2 (P2) results, which included 1% *Spirulina* sp. in 100g of feed. The following bar depicts Treatment 3 (P3), with 3% *Spirulina* sp. added to the feed, and the final bar on the right illustrates Treatment 4 (P4), which had 5% *Spirulina* sp. in the feed.

According to the Toca Colour Finder (TCF) results presented in Fig 3 there were no significant changes observed in Treatment 1 (control) from the first week to the fourth week of the study. This indicates that the feed provided ad libitum without *Spirulina* sp. addition, which did not enhance color quality. Test animals in P1 showed consistent color intensity throughout the study period.

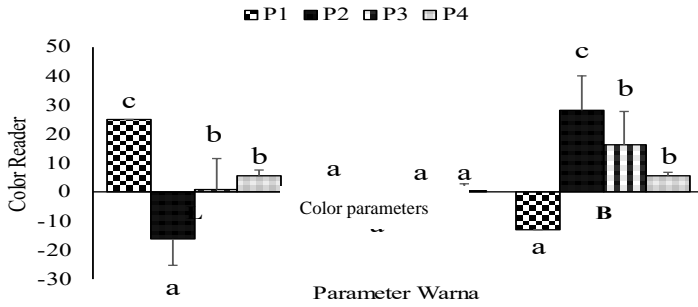


Fig 3. LAB value measurement using a color reader

In contrast, P2 exhibited the highest color improvement among all treatments, including the control and higher concentrations of *Spirulina* sp. P2 saw an increase of 2.6 units, from a starting value of 19 in the first week to 21.6 in the fourth week. This indicates that adding 1% *Spirulina* sp. to the feed positively and significantly affects color enhancement in test animals.

P3 showed an increase of 1.3 units from the first week to the fourth week, with values rising from 18 to 19.3. Although this increase is not as high as that of P2, it demonstrates a notable effect of 3% *Spirulina* sp. on color improvement.

Treatment 4, with 5% *Spirulina* sp., had a more minor color increase than the other treatments, with values moving from 19 to 19.3 over the study period. Thus, while 5% *Spirulina* sp. influenced color improvement, it was less effective than lower concentrations.

After evaluating color changes using TCF, a final analysis was performed using the Color Reader CR-10 with CIELAB 1976 calculations. Initial comparisons were made against the standard control values (Treatment 1), with each value (L, A, and B) from Treatments 2, 3, and 4 subtracted from the control values. The resulting data will be analyzed further using the delta E (ΔE) formula, as detailed in the following table.

$$\Delta E = \sqrt{L^2 + A^2 + B^2} \quad (6)$$

The principle of the Color Reader is based on the CIE color space. The L, A, and B indicators are commonly used to measure color in fish. The LAB color space is utilized for this analysis, with delta E combining L, A, and B values using the CIELAB 1976 formula. According to Safitri (2014), the Color Reader parameters are as follows: L: Indicates lightness, with values ranging from 0 (black) to +100 (white). A: Represents color dimensions from green (negative) to red (positive). B: Indicates color dimensions from blue (negative values) to yellow (positive values).

In the study conducted by Sari [17] the CIELAB color structure is explained as follows: L: A value of 0 represents black, while +100 represents white. A: Negative values indicate green hues and positive values indicate red hues. B: Negative values represent blue hues, while positive values represent yellow hues after obtaining results from the Complete Random Design (RAL).

Although no significant differences were observed in ΔE (delta E), as stated in the study and analysis by Sari [19], color analysis can be conducted using the dimensions *L, *A, and *B. According to the previously presented table, significant differences were noted in the dimensions *L and *B among the treatments. Therefore, further analysis using Duncan's Multiple Range Test (DMRT) at a 5% significance level will be conducted to identify the treatment that provides the most substantial improvement in color. The DMRT in this study

will be applied to the dimensions that have been shown to differ significantly, as outlined in the following Fig 3.

The results from (DMRT) indicate no significant differences between Treatment 3 and Treatment 4, as both are categorized with the letter 'b'. Additionally, a notable effect was observed between P1 and P3. The table shows that P2 has a significant effect compared to Treatment 1, demonstrating a statistically significant difference.

The results from the (DMRT) for dimension *B, which indicates yellowish coloration in the fish, reveal that there are no significant differences between P3 and P4, both being categorized with the letter 'b'. However, there is a significant difference between P1 and P4, although it is not significant. According to the table, P2 shows a significant difference compared to Treatment 1.

Based on the descriptive statistics graph from the Colour Reader, P2 with a 1% *Spirulina* sp. concentration added to the artificial feed exhibits a significant color difference compared to P1 (control), relative to other treatments, in both dimensions *L and *B as per the LAB Hunter in CIELAB 1976.

The observations indicate that during the first and second weeks of maintenance, there was no significant color enhancement in Betta fish as measured by the modified Toca Colour Finder (TCF). This lack of improvement is attributed to the fish adapting to the changes. However, color enhancement was observed in the third and fourth weeks, marking the study's end. The final Color Reader analysis revealed that all treatments influenced the dimension *L, indicating brightness, and dimension *B, indicating yellow coloration, with significant differences. The delta E and dimension *A, indicating blue and red hues, did not show significant differences. Treatment 2, with a 1% *Spirulina* sp. concentration, demonstrated the highest color enhancement compared to the other treatments based on the indicators L and B. This suggests that a *Spirulina* sp. concentration of 100g/1kg of artificial feed is optimal for improving color intensity in Betta fish (*Betta splendens*). LAB measurements and analysis results have the same level of measurement accuracy or do not differ between treatments as shown by the ΔE value in Fig. 4.

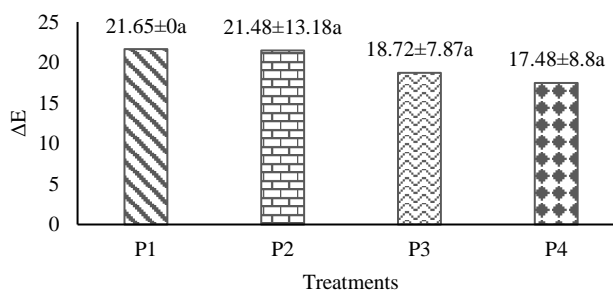


Fig. 4. Measure of change in visual perception of two specific colors (ΔE) in each treatment. These findings align with previous research by Sudirman [20], which reported that a 1% *Spirulina* sp. concentration enhances color in koi fish (Kohaku) through carotenoid intake. The study also corroborates [13], who found that 1% *Spirulina* sp. in paste form enhances red coloration in koi fish. Treatments 3 and 4 did not achieve the color quality observed in Treatment 2, likely due to excessive carotenoid levels in the feed, leading to suboptimal color brightness. Treatment 1, without *Spirulina* sp. addition, showed insignificant color improvement, suggesting that the feed might contain other carotenoids affecting fish coloration. According to Subamia [21], the intensity of fish color is enhanced by the natural carotenoids in *Spirulina*

sp. Excessive carotenoids in the feed may lead to varying color outcomes due to differences in fish carotenoid absorption capabilities.

3.1.1. Growth of Absolute Weight

The growth of absolute Weight is a supporting parameter in the study to assess the impact of Spirulina sp. addition to the artificial feed on Betta fish growth. Growth was measured based on the Weight and length of the fish, as detailed in Fig 5.

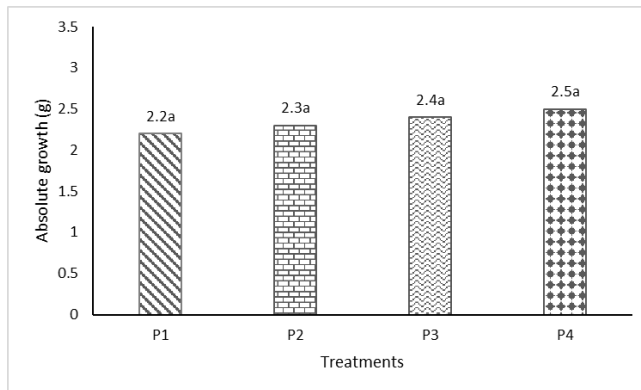


Fig. 5. Absolute weight growth in fish with and without treatment

Growth measurements of absolute Weight were conducted at the end of the study using the previously described formula: Weight at the end of observation minus the initial Weight. The table shows that P1 exhibited significant weight growth compared to other treatments, followed by P3, P2, and P4 with the least weight growth.

The data indicate a significant difference in weight growth among treatments, with the highest growth in P1 and the lowest in P4. Factors affecting fish growth include both external and internal factors. Spirulina sp. is a nutrient-rich algae containing high levels of vitamins, fats, carbohydrates, and proteins. Fish require 20-60% protein, with optimal levels being 30-36% [20]. Previous research by Koru [22] reported that Spirulina sp. contains 60-70% protein in its dry Weight. Thus, the lower growth in Treatment 4 is likely due to excessive protein levels, leading to protein accumulation in the digestive tract and poor nutrient absorption [23]. Excessive protein can lead to higher energy requirements for metabolism. The addition of carotenoids to the feed did not affect ornamental fish growth significantly, possibly because fish prioritize carotenoids for color enhancement rather than [8] found that astaxanthin carotenoid supplementation via Daphnia improves color in Halfmoon Betta fish [11]. Quality feed and its impact on growth depend on the fish's ability to utilize and respond to the feed effectively [24].

3.1.2. Growth of Absolute Length

The growth of absolute Weight is a supporting parameter in the study to assess the impact of Spirulina sp. addition to the artificial feed on Betta fish (*Betta splendens*) growth. Growth was measured based on the Weight and length of the fish, as detailed in the table Fig. 5 below.

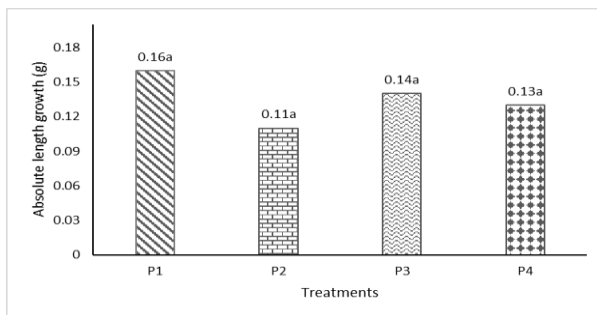


Fig. 6. Absolute length growth in fish with and without treatment

According to Isnawati [25], feed affects fish growth and development by providing energy and enhancing reproduction and movement. Consumed feed contributes to tissue building and growth stimulation. *Spirulina sp.* accumulates 55-70% protein in its dry [20]. Therefore, the lower growth in Treatment 4 is likely due to excessive protein levels, affecting enzyme function and nutrient absorption, leading to reduced energy for growth [25].

3.1.3. Water Quality

The aquatic environment is crucial for aquatic organisms as it influences fish stamina. Unhealthy fish may use feed energy solely for maintenance, leading to suboptimal growth and color enhancement. The water quality observations compared to standard Betta fish water quality are detailed in the table below.

Table 1. Water Quality

Water quality	P1	P2	P3	P4	Ref. [11]
DO	8	8	8	8	5-12
pH	7.2	7.5	7	6.8	6.5-7.5
Suhu	28	28	27	29	24-30

Water quality measurements during the study showed that the average values were within the recommended standards for Betta fish, with Dissolved Oxygen (DO) at eight across all treatments and pH values of 7.2 for P1 and P2, 7 for P3, and 6.8 for P4. Temperature measurements were 28°C for P1 and P2, 27°C for P3, and 29°C for P4. The water quality during the maintenance period was considered suitable for Betta fish. Water quality impacts metabolism and feeding rates. A water temperature range of 22-30°C is optimal for fish cultivation [11].

4 Conclusion

Based on the research findings, adding *Spirulina* powder at 1g/100g significantly enhances color intensity, as indicated by a more reddish-yellow hue observed in weeks 3 and 4.

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