

## THE EFFECTIVENESS OF SUPPLEMENTATION OF *SPIRULINA* SP AND ASTAXANTHIN WITHIN FEED TO COLOR QUALITY OF COMET FISH (*CARASSIUS AURATUS*)

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### ABSTRACT

The purpose of this study was to analyze the effect of artificial feeding with the addition of a combination of *Spirulina* sp. and astaxanthin on the color quality of comet goldfish (*Carassius auratus*) seeds. The method used in this research was to apply a combination of dosages of *Spirulina* sp. and astaxanthin in artificial feed, namely 0% *Spirulina* sp.:0% Astaxanthin; 1% *Spirulina* sp.:0.1% Astaxanthin; 3% *Spirulina* sp.:0.1% Astaxanthin; and 5% *Spirulina* sp.:0.1% Astaxanthin. Experiments using a completely randomized design (CRD) consisted of four treatments and three replications, the color of the fish in each treatment was observed using a modified *Toca Color Finder* (TCF). The results of this study also indicate that the addition of 1% *Spirulina* sp. and 0.1% astaxanthin gave the best color to comet goldfish seeds.

Keywords: Comet Goldfish, Astaxanthin, *Spirulina* sp., Color Quality, Growth

### INTRODUCTION

Comet-tailed goldfish (*Carassius auratus*) is one of the most popular species of ornamental freshwater fish that lives in shallow waters. Comet fish is popular to the public because of the attractiveness of various colors such as white, orange, red, black, brown or a combination of these colors such as white orange or black orange, thus making comet fish have a high commercial value, and many people are attracted to make a profit from this fish (Lingga and Susanto, 2003). Color of this fish often changes due to changes in the amount of pigment. One of the causes is environmental stress, such as sunlight and water quality. Foods also have an influence in the formation of ornamental fish color (Irianto, 2005).

The content of pigment cells in the body of fish is enriched through feeding containing astaxanthin (Sitorus et al., 2014). Astaxanthin is an effective carotenoid pigment and is widely used for coloring ornamental fish because fish will absorb it from feed and use it directly as red pigment cells (Meiyana and Minjoyo, 2011). *Spirulina* sp. may be used as an additional feed ingredient for comet fish because it contains about 40-60% protein, vitamin A, 3-7% minerals and beta-carotene (Rosid et al., 2019). *Spirulina* sp. rich in beta carotene so that it may increase the red color (Sunarno, 2012). From the composition of the ingredients contained in astaxanthin and *Spirulina* sp. the researchers tried to conduct a study to determine the effectiveness of the combination of astaxanthin and *Spirulina* sp. on artificial feed on the color quality of comet fish (*Carassius auratus*).

### MATERIALS AND METHODS

This research was conducted from December 2020 to January 2021, at the Fish Development Laboratory, Faculty of Fisheries and Marine Sciences, Mulawarman University. The method used in this study was a completely randomized design (CRD) method with the following treatments:

Table 1. Treatments used during the study

Treatment	Astaxanthin	<i>Spirulina</i> sp.
1	0%	0%
2	0,1%	1%
3	0,1%	2%
4	0,1%	3%

The tools used in this study were an aquarium measuring 80x40x40 cm<sup>3</sup> (6 units), aeration stone, aeration hose, biofoam, blower, tray, spoon, basin, plastic clip, 5 ml syringe, 1 ml syringe, 15 ml test tube, beaker glass and weighing scale with an accuracy of 0.01 g, ruler, scoop, plastic tub with a volume of 82 liters (12 pieces), Measurement of water quality: Oxygen meter (0.1 mg/L), pH meter Ezdo (0.01), Oxygen meter (0.10C), Spectrophotometer Taomsun (0.001), a modified *Toca Color Finder* (TCF), a nikon D3100 camera equipped with a nikon AF-S Nikkor 50mm F/1.8G lens and a computer equipped with Adobe Photoshop 7.0 software. The fish used were comet fish seeds obtained from spawning carried out in the Fish Development Laboratory, Faculty of Fisheries and Marine Sciences, Mulawarman University with an average weight of 0.07 g, length +0.9-1.7 cm and age +30 day. The feed ingredients are water, factory feed in the form of flour brand Hi-Pro-Vite PSP (Pre-Starter-Pasta) with a protein content of ±37% produced by PT. Central Proteina

Prima Tbk, *Spirulina* flour. Mackay Marine's Microfine *Spirulina* brand, Carophyll Red 10% astaxanthin flour produced by DSM Nutritional Product France SAS. Materials to measure the quality of ammonia water were distilled water, phenate reagent, MnSO<sub>4</sub>, clorox, and 1 ppm (mg/l) standard ammonia.

### Research Procedure

During the acclimatization process, the fish were fed twice a day in the form of a paste made from fish meal enriched with *Spirulina* sp. as much as 2% as an adaptation of comet fish (*Carassius auratus*). Fish seeds were sorted every day according to their size with an acclimatization process for one month. The siphoning process was carried out every day in the morning and new water was filled in each container to replace the water wasted during siphoning. After the siphoning activity, feeding activities were carried out in the form of pasta dough on fish seeds with the frequency of feeding three times a day on an ad satiation basis, namely at 9am, 1pm and 5pm and siphoning in the rearing container. The feed used during the study was home-made feed with the composition of raw materials according to the dose in each treatment. The first stage was to mix fish meal with *Spirulina* sp flour with a dose of 100 grams, then the results of the feed mixture were taken as much as 5 grams and then mixed with astaxanthin flour that has been dissolved in water as much as 10 ml, the feed raw materials were mixed to form a dough and then given to the fish seeds.

### Data Collection and Processing

#### Color Changes

Color observation was carried out by calculating the color intensity following Barus et al. (2014), by comparing the increase in color in each treatment and replication by observing the color changes in the sampled fish and the increase in weight in the M-TCF. Observation of the color change of comet fish was done by giving a value or weighting based on color measuring paper. The assessment starts from the smallest score of 1 to the largest score of 30 with color gradations from light yellow to dark red.

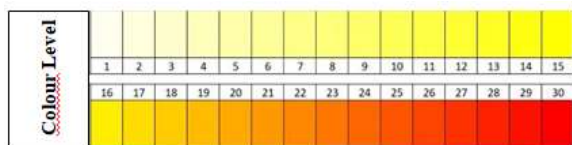


Figure1. M-TCF

### Color Variation

Observations of color variations were carried out by grouping fish based on the type of color in each treatment and replication and then counting the number of color variations. The total value of each color variation contained in the subsequent treatment was presented as a percentage starting from the dominant color variation to the least color variation. Percentage of color variation using the following formula

$$\%Color = \frac{NV}{NT} \times 100\%$$

Where:

- %Color = Percentage of color at each treatment (%)
- NV = Number of fish at each color variation
- NT = Number of fish at each treatment

The supporting data observed in this study were water quality measurement data. The water physicochemical parameters measured were dissolved oxygen (DO), pH, temperature and ammonia (NH<sub>3</sub>).

## RESULTS AND DISCUSSION

### Color Quality and Variation of Comet Fish (*Carassius auratus*) Seeds

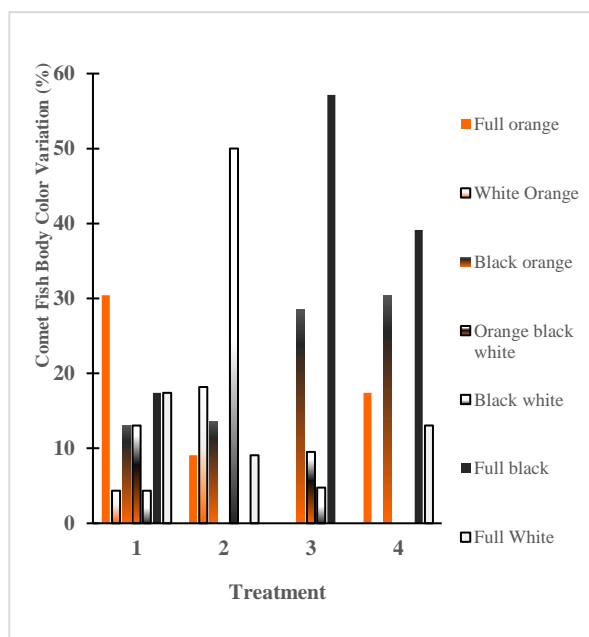


Figure 2. Color variation of comet fish (*Carassius auratus*)

Research on feeding combination of *Spirulina* sp. and astaxanthin with different doses in each treatment that has been carried out for 60 days on the color quality of the comet fish (*Carassius auratus*) fry, the results are shown in Figure 2.

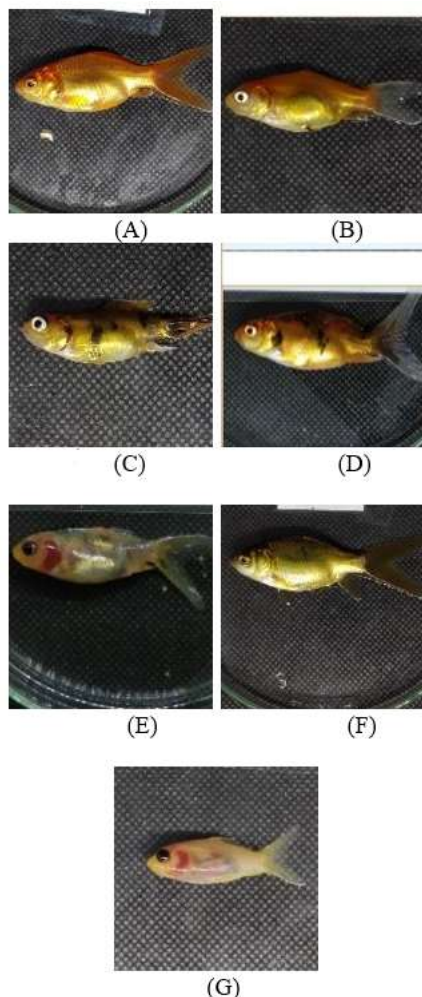


Figure 3. Body Color of the Comet Fish (*Carassius auratus*)

Description:

(A). Full orange; (B) . White orange; (C) Black orange; (D). Black and white orange; (E). Black and white; (F). Full black; (G). Full white.

Figures 2 and 3 indicate that the application of *Spirulina* sp and Astaxanthin in artificial feed resulted in body color quality which tended to vary and differ in each treatment. In the P1 treatment (control, without the addition of astaxanthin and *Spirulina* sp), the highest percentage produced was full orange with a value of 30%, followed by full black and black and white with a percentage of 17%. The percentage of color variation that appears the least in the P1 treatment is orange and white and black and white with a value of 4%. In the P2 treatment (combination of astaxanthin 0.1% and *Spirulina* sp 1%), the highest percentage of variation produced was black and white with a value of 50% and orange and white with a value of 18% and the percentage of color variation with the lowest value was full orange and full white with a value of 9%.

Table 2. Percentage of Color Variation

	P1 (%)	P2 (%)	P3 (%)	P4 (%)
Full orange	30	9	0	17
White orange	4	18	0	0
Black orange	13	14	29	30
Black-white orange	13	0	10	0
Black and white	4	50	5	0
Full black	17	0	57	39
Full white	17	9	0	13

In the P3 treatment (combination of astaxanthin 0.1% and *Spirulina* sp 3%), the highest percentage results were full black with a value of 57% followed by black orange with a percentage value of 29%, orange black and white 10%, while the percentage the lowest color variation is black and white with a value of 5% and the color variations that do not appear in this treatment are full orange, white orange and full white.

In the P4 treatment (combination of astaxanthin 0.1% and *Spirulina* sp. 5%) resulted in 4 color variations with the dominant color appearing is full black with a percentage of 39% and black orange color with a value of 30% and the color variations that appear the least are full white color with a percentage value of 13%.

Overall, the results of the study in each treatment showed that the dominant fish color appeared, i.e., full black found in treatment P3 (57%) and treatment P4 (39%), followed by full orange color that appeared in treatment P1 (30%) and treatment P3 (17%), and the black orange color found in treatment P3 (29%) and treatment P4 (30%). While the color variations with the lowest percentage values are orange white and black and white in treatment P1 (4%), full orange and full white in treatment P2 (9%), black and white in P3 (5%) and full white in P4 (13%).

The treatment that has the most color variations is treatment P1 with 7 color variations, treatment P2 produces 5 color variations, treatment P4 and P3 produce 4 color variations. The movement of pigment granules in clusters or scattered in the color pigment cells as a result of different stimuli such as temperature, light and others. Based on the results of observations, dark colors tend to fade such as white and black which are found in each treatment, especially the dominant ones appear in treatment P2, treatment P3 and treatment P4 allegedly because fish tend to produce melanophore pigment cells, i.e., pigments that store black color (melanin), leucophores store white pigment and iridophores that do not contain pigment except guanine crystals that are able to reflect light into their constituent color components. In addition, pigment grains that collect near the nucleus cause a decrease in the color of the scales so that the color of the fish looks darker and fades (Anderson, 2000; Sally, 1997).

In this observation, the resulted color variations are orange, white, and black. The dominant black and white color appears are caused by chromatophores cells that contain pigment under endocrine control, chromatophores may change the distribution of pigment in pigment cells (collected or scattered) in minute size or not (Isnaeni, 2006). This lack of optimal color variation is influenced by the levels of carotene contained in the feed given are higher than the required carotene levels (Bachtiar, 2002), causing the absorption and metabolism of koi fish to carotenes is not optimal and causes color brightness in fish. does not reach the maximum. According to Utomo et al. (2006), the requirement for carotenoids in young fish is relatively less because the change in body color is not well developed. The fish used are two months old with an average weight and length of 35 g and 13 cm. This study used mass spawned seeds with parent color ranges of orange, white and black.

In addition to the factor of feed, environmental factors also affect the optimal color variation. According to Sulawesty (1997), environmental factors affect color changes and the appetite of the cultured fish. The living environment of fish is water, so water quality needs to be considered. P1 which is the control, namely feed without the addition of a combination of *Spirulina* sp. and astaxanthin still produce a dominant orange color. This is due to the source of  $\beta$ -carotene contained in fish flour (Satyani and Sugito, 1997).

### Level of Color Brightness

The results of observations on the level of color brightness, especially the orange color found in the comet fish (*Carassius auratus*) seed, by comparing the original color of the fish to the modified and weighted TCF (Toca Color Finder) color measuring paper, showed results as shown in Figure 4.

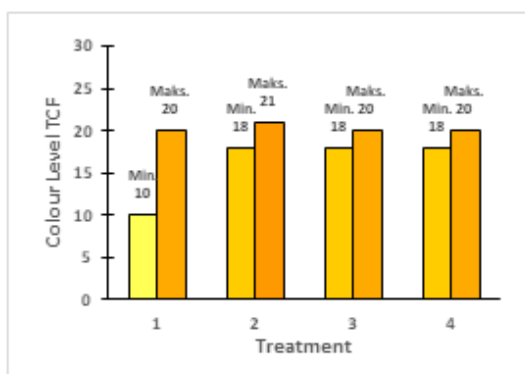


Figure 4. Level of color brightness of comet fish

The results of the observations on the brightness level of the comet fish (*Carassius auratus*) seed informed that the highest color level increase was found in

treatment P2 with a range of TCF color levels of 18 to 21, followed by treatment P3 and P4 with a range of TCF color levels of 18 to 20, while the lowest color level was found in treatment P1 with a color level range of TCF 10 to 20.

In the P1 treatment, the color brightness of the comet fish (*Carassius auratus*) is located at the TCF color level of 10 to 20. This is presumably because the P1 treatment as the control (treatment without the addition of *Spirulina* sp. and astaxanthin) causes the color of the fish to become more pallid. Bachtiar (2002) stated that the pigment content in the feed is one factor that affect the brightness of fish color. This is supported by the statement of Gunawan (2005) that the increase in color in the control treatment is estimated because in the feed there is fish f containing  $\beta$ -carotene which indirectly affects color changes in fish.

In the P3 and P4 treatments, the similar results of TCF color levels of 18 to 20. While in treatment P2, the results of the TCF color level ranges of 18 to 21. Treatment P2 produces a high level of color brightness. This better level is suspected due to the addition of a combination of *Spirulina* sp. and astaxanthin in feed contains carotenoids that can be absorbed and utilized by comet fish seeds optimally.

This is in accordance with the statement of Satyani et al. (1997) that the supplementary of carotene into the feed has a maximum limit, meaning that if carotene is added to the feed in excess at a certain condition will not give a better color change, it may even reduce the color value. Value of the brightness contained in the feed given was higher than the level of carotene needed, causing the absorption and metabolism of koi fish to carotenes was not optimal and caused the brightness of the color of the fish did not reach the maximum (Bachtiar, 2002), and according to Amin et al. (2012), the occurrence of different color increases in each treatment was due to fish having different absorption rates for the type of color pigment and the dose given.

Based on the observation results, P2 treatment is a treatment with the addition of 0.1% astaxanthin and *Spirulina* sp. 1% produces the best level of color brightness, this is in accordance with the statement of Utomo et al. (2006) that the addition of 1% of *Spirulina platensis* to feed resulted in the highest level of color change in koi fish (*Cyprinus carpio* L) and was more effective than other *Spirulina* levels, supported by the statement of Bachtiar (2002) the level of carotene in accordance with the needs may directly be absorbed entirely by pigment cells in koi fish optimally because the levels given are adequate to the fish's ability to synthesize carotene. The content of  $\beta$ -carotene in *Spirulina* sp flour will be utilized by fish, it may be observed by the change in color of the fish, the higher the addition of *Spirulina* flour, the increase in brightness of fish color is higher (Nafsihi, 2016) but this did not occur in the observation of the color quality of the comet fish (*Carassius auratus*) because the observation process used a combination of astaxanthin and *Spirulina* sp.

## Water Quality

The factors that determine the color quality of ornamental fish are the quality of koi (70%), water (20%), and other factors (10%) (Gomelsky et al., 2003). In the rearing of comet fish (*Carassius auratus*) the temperature range is between 26-29 °C, this temperature is considered suitable to the needs of the comet fish, i.e., 26-30 °C (SNI 8110, 2015). Dissolved oxygen levels ranged from 2.2 mg/L to 7.3 mg/L, at rearing comet fish the range of dissolved oxygen required according to Rahmadiyah (2013) in Wihardi et al. (2014) the dissolved oxygen content for rearing comet fish is 4.

Chemical processes in water are determined by the value of the degree of acidity (pH) of water because a pH that is too acidic or alkaline causes fish to become stressed (Sumantri et al. 2017). Changes in pH that are too large and occur continuously may inhibit growth

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## CONCLUSION

The treatment with the most color variations is P1 treatment with 7 color variations, P2 treatment produces 5 color variations, P3 and P4 treatments with 4 color variations. In this observation, the resulting color variations are orange, white, and black.

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