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## 2 Effect of partial replacement of fish meal with *Spirulina platensis* meal in practical diets and culture location on growth, survival, and color enhancement of percula clownfish *Amphiprion percula*

S Hudaidah<sup>1\*</sup>, B Putri<sup>1</sup>, S H Samara<sup>2</sup> and Y T Adiputra<sup>1</sup>

<sup>1</sup>Aquaculture Department, Faculty of Agriculture, Lampung University, Lampung, Indonesia

<sup>2</sup>Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, East Java, Indonesia 60115

\*Corresponding author: [siti.hudaidah@fpunila.ac.id](mailto:siti.hudaidah@fpunila.ac.id)

**Abstract.** This experiment was aimed to understand the effect of partial replacement of fish meal with *Spirulina* meal in percula clownfish *Amphiprion percula* diet on its growth, survival, and color enhancement. One control (A) and three *Spirulina* dosage were used in this 60-day experiment: 0.9% (B), 1.2% (C), and 1.5% (D) with three replicates for a total of 12 aquariums. During the experiment, the clownfish were fed 4 times daily with 5% FR and periodic sampling for growth monitoring. No significant difference was found in the color intensity, absolute length, and absolute weight among all treatments. On the other hand, survival was the highest on 1.2% *Spirulina* meal addition to feeding.

### 1. Introduction

Clownfish are one of the tropical fishes found in many places in Indonesia. With a high economic value, it is highly sought by ornamental fish breeders[1–3]. Among them, Percula clownfish *Amphiprion percula* is easily identified by its orange color with three stripes where the middle stripe stands out to the front and varying black lining on the fins. Such striking color is coming from carotenoid, a pigment synthesized by all photosynthetic organisms and fungi, including microalgae[4,5]. The color intensity of fish is affected by internal factor e.g. age as well as external factors e.g. water quality, light intensity, and feed containing high carotene and nutrition. It will increase until a certain age and decrease afterward, thus carotenoid is needed to preserve it [6].

As it could not be composed by the fish, carotenoid must be supplied outside the fish[7]. *Spirulina*. the meal is a good carotenoid source that gives the fish its distinct yellow and red color with 60-70% protein and 1,5-12% lipid[5,8]. Aside from the protein content, *Spirulina* contains phycocyanin, a blue pigment function as protein reserve and antioxidant [9]. The pigment helps in non-specific immune system modulation by increasing the number of blood erythrocyte and leukocyte, which in turn distributes nutrient and oxygen efficiently throughout the body [10].

The present work was undertaken to understand the effect of *Spirulina* sp. meal added to the fish meal on color enhancement, growth performance, and survival rate of percula clownfish reared in indoor and outdoor tanks.

## 2. Material and method

### 2.1 Experimental design

The study was conducted at the Research Center and Development of Marine Aquaculture, Pesawaran, Lampung Province, Indonesia. This experiment used 3 treatments and 1 negative control: no *Spirulina* on feed (A), 0.9% *Spirulina* (B), 1.2% *Spirulina* (C), 1.5% *Spirulina* (D) with three replications so a total of 12 aquariums were used.

### 2.2 Method

#### 2.2.1 Experimental diets

The clownfish were fed with feed containing fish meals, soy meal, corn meal, *Spirulina* meal, wheat flour, fish oil, corn oil, and premix. The dry ingredients were finely milled, weighed according to the prescribed formulation, and homogenized. The mixture was then molded and dried for approximately 12 hours prior to entering the oven. Lastly, a proximate analysis was performed to determine the nutrient within the feed (Table 1).

**Table 1.** Raw material formulation (%) and proximate analysis of experimental diets for percula clownfish *A. percula*

Ingredient	Composition (%)			
	A (control)	B	C	D
Fish meal	49,53	48,63	48,33	48,03
<b><i>Spirulina</i> sp. meal</b>	<b>0</b>	<b>0,9</b>	<b>1,2</b>	<b>1,5</b>
Corn meal	24,76	24,76	24,76	24,76
Soy meal	13,71	13,71	13,71	13,71
Tapioca	7	7	7	7
Fish oil	2	2	2	2
Corn oil	1	1	1	1
Premix	2	2	2	2
Total	100	100	100	100

Experimental Diet	Proximate Parameter			
	Protein (%)	Carbohydrate (%)	Lipid (%)	Fiber (%)
<b>A (control)</b>	34,17	36,13	3,45	2,35
<b>B</b>	33,33	36,78	3,44	2,04
<b>C</b>	37,91	32,60	3,11	1,58
<b>D</b>	36,70	33,27	4,22	1,57

#### 2.2.2 Feeding trial

Clownfish with an initial length of 2-3 cm was reared in 30 fish/aquarium density for 60 days. Prior to the experiment, the clownfish were acclimatized for 7 days. During the experiment, the feed was given at 8 AM, 10 AM, 2 PM, and 4 PM with 5% FR. Water was maintained by exchanging every 10 days prior to feeding and siphoning the uneaten feed and feces. Temperature and pH were checked daily, salinity every 10 days, while ammonia and nitrite were measured at the start, middle, and end of the experiment.

#### 2.2.3 Performance evaluation

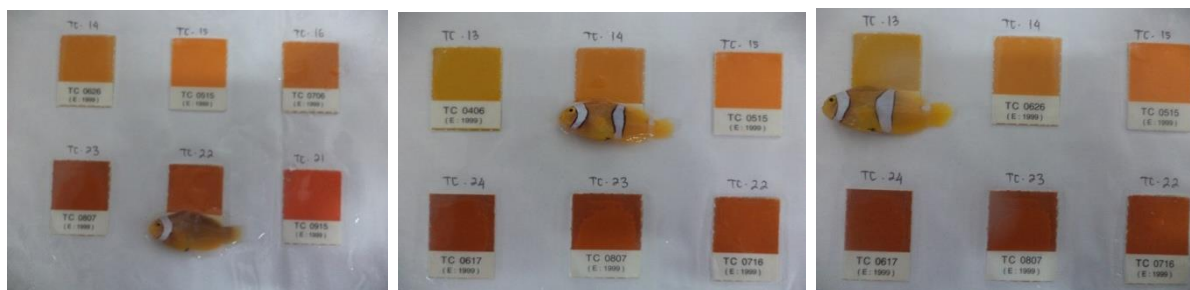
The growth in length and weight were monitored every 10 days at 8 AM. For the color intensity parameter, the body color was scored compared to the M-TCF (*Modified Toca Color Finder*). The color was observed by 5 people with normal vision at the beginning and the end of the experiment. Chromatophore histology analysis was also performed at the beginning and the end of the experiment.

#### 2.2.4 Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) and BNT multiple comparison tests using a statistic program of SPSS version 21 for Windows. All probability values were set on 0.05 level of significance. Chromatophore analysis and water quality were descriptively analyzed.

### 3. Results

The increase in color intensity was measured by comparing the fish color to M-TCF, where the result was later quantified. A lower M-TCF score indicates a more vivid body color. The experiment showed an increase in color intensity (Figure 1). The increase started at day-30 as the fish started to adapt to the experimental feed. At day-40 the control fish showed no change in color intensity as opposed to the treatments. The color intensity is highest when 1.2% and 1.5% *Spirulina* meal was added to the feed, yet the difference was not significant ( $p>0.05$ ). High carotenoid on fish skin rose with the increasing carotenoid content of the feed, causing a richer color intensity on fish [11].



**Figure 1.** M-TCF method to assess percula clownfish (*Amphiprion percula*) color enhancement

**Table 2.** Growth, Survival, and Color Enhance of Percula clownfish cultured outdoor and indoor

Treatments	Outdoor Culture			Indoor Culture		
	Growth (total length-body weight) (cm-g)	Survival (%)	Color Enhance Score (start-final)	Growth (total length- body weight) (cm-g)	Survival (%)	Color Enhances Score (start-final)
No <i>Spirulina</i> meal	3.13±0.13 <sup>a</sup> (TL) 0.75±0.08 <sup>a</sup> (BW)	96.67±0 <sup>a</sup>	16±0.08 - 14±0.08 <sup>a</sup>	3.16±0.07 <sup>a</sup> 0.71±0.06 <sup>a</sup>	96±5.1 <sup>ab</sup>	14.5±0.89 - 13.97±0.89 <sup>a</sup>
<i>Spirulina</i> meal 0.9%	3.18±0.11 <sup>a</sup> 0.79±0.05 <sup>a</sup>	98.89±1 <sup>ab</sup>	16±0.05 - 14±0.05 <sup>a</sup>	3.18±0.09 <sup>a</sup> 0.71±0.06 <sup>a</sup>	94.44±5.1 <sup>a</sup>	14.4±1.01 - 14.01±1.02 <sup>a</sup>
<i>Spirulina</i> meal 1.2%	3.25±0.14 <sup>a</sup> 0.82±0.04 <sup>b</sup>	100±0 <sup>b</sup>	16±0.04 - 14±0.05 <sup>a</sup>	3.31±0.07 <sup>c</sup> 0.80±0.02 <sup>c</sup>	98.89±1.9 <sup>a</sup> <sup>b</sup>	15.2±1.26 - 14.46±1.26 <sup>a</sup>
<i>Spirulina</i> meal 1.5%	3.19±0.09 <sup>a</sup> 0.75±0.05	100±0 <sup>b</sup>	17±0.05 - 13±0.05 <sup>a</sup>	3.23±0.03 <sup>b</sup> 0.76±0.01 <sup>b</sup>	100±0 <sup>c</sup>	15.6±1.14 - 14.39±1.14 <sup>a</sup>

Note. Growth, survival and color enhance of percula clownfish (*Amphiprion percula*) with the partial addition of *Spirulina platensis* meal cultured in different locations. Means in a column with different letters were significantly different ( $P < 0.05$ ).

### 4. Discussion

Color intensity in fish was affected by either internal or external factors. Internal factors included fish age, size, genetics, sex, and nutrient absorption from the feed, while external factors include water quality, light, and feed high in nutrition and carotenoid [6]. The color change in this experiment might be contributed not only by the *Spirulina* but also from the  $\beta$ -carotene in fish meal [12].

Chromatophore, around pigment cell in every layer of the fish epidermis contributed to the vividness and sharpness of the body color and patterns. A brighter color was a result of the chromatophores concentrating near the nucleus, reducing the color intensity on the scale. The chromatophore analysis showed that adding 1.2% *Spirulina* meal to the feed increased the chromatophore cell number up to 746 cell/sample compared to the control of 182 cell/sample.

*Spirulina* meal addition to feed, however, had no significant effect on absolute length and absolute weight. For the survival rate, deaths were found on the control fish due to bacterial disease. Symptoms were alienation from the group, pale body and organs as well as swelling of liver and kidney. The phycocyanin in *Spirulina* had immunostimulant properties, triggering the production of red blood cells[13]. Even the location did not affect significantly since the clown fish could be maintained in the outdoor or indoor [2, 5].

When added to percula clownfish feed, 1.2% *Spirulina* sp. meal has a significant effect on fish survival, but not on color enhancement and growth performance. Future experiments on *Spirulina* sp. meal

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