

Formulation of Feed with Different Source of Carotenoids on the Colors Quality of Sunkist Balloon Molly Fish (*Poecilia* sp.)

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Abstract

Received : 2022-08-25 Accepted : 2022-11-30

Keywords : Color quality, Feed formulating, Molly fish

The parameter value of ornamental fish is determined by the brighter the color of a type of ornamental fish, the higher the value so farmers need to maintain the color of the ornamental fish by providing feed containing color pigments that can be obtained from vegetable and animal sources of carotenoids. Sunkist balloon molly fish (Poecilia sp.) were stocked in an aquarium measuring $60 \times 30 \times 30$ cm (5 fish/l), Objective of this study was to determine the effect of feeding formulations using different carotenoid sources with the best treatment for improving the color quality of Sunkist balloon molly fish (Poecilia sp.). The method used was an experimental (quantitative) completely randomized design (CRD) with 3 treatments, 1 control, and 3 repetitions. Feed was given ad libitum (5% body weight) 3 times a day. Color quality data were analyzed using ImageJ Software and a one-way ANOVA test. The results of the study showed that feed utilization efficiency, feed conversion ratio, and growth performance were not significantly different between treatments. There was a significant effect on improving the quality of the color shown in the P_3 (vellow pumpkin) treatment of 10% as the best treatment among others. Water quality parameters observed trend to be normal and stable from the beginning to the end of the maintenance period.

INTRODUCTION

The beauty and uniqueness make ornamental fish favored by the people in fish farming, until now the fish farming who initially farming consumption fish changed professions to become freshwater ornamental fish farming (Lesmana and Iwan, 2012). A large market probability, both for local and export market centers for ornamental fish sales. Ornamental fish can be farmed in large ratios or small ratios of households and the initial capital turnover is relatively very fast, within two years (Soebiakto, 2014). Sunkist balloon molly fish (*Poecilia* sp.) comes from Mexico, but currently, the existence of this fish can be found anywhere, including in Indonesia (Koutsikos *et al.*, 2018). Sunkist balloon molly fish (*Poecilia* sp.) is one of the other ornamental fish in great demand by freshwater ornamental fish lovers,

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because of its bright orange color and unique round body shape, so it exudes its charm. The survival of this fish is about 1.5 years, ovoviviparity reproduction (5 months), and is omnivorous (Froese and Pauly, 2014).

An important parameter in measuring the level of marketability of ornamental fish is the color produced from chromatophores cells on the skin of the epidermis, the brighter the color quality level of a type of ornamental fish, the higher the selling power, thus the brightness level of the color needs to be maintained by farmers (Novivanti et al., 2015). Carotenoids are the main pigments in the skin of ornamental fish, but fish cannot synthesize them. Thus, the input of additional carotenoids must be provided through artificial feeds that are formulated with carotenoid sources (Ahilan and Nithiyapriyatharshini, 2015).

Currently, many carotenoid pigments in the feed are found at relatively expensive prices because in the market only synthetic color pigments are found which produce color changes quickly and instantly, but if accumulated in the long term it will harm the fish body and cause environmental damage (Setyogati, 2021). Based on this, it is very necessary to have innovations in the use of natural materials (vegetable and animal) as an alternative to materials that are easily available and at economical prices. This study aims to determine the effect of feeding formulations with different carotenoid sources on improving the color quality of Sunkist balloon molly fish (Poecilia sp.) and evaluating the best dosage of feed formulations to improve color quality.

Research using a combination of different carotenoid sources, such as marigold flowers (*Tagetes erecta L.*), shrimp head waste, and pumpkin (*Cucurbita moschata D.*) has proven successful and has been carried out by previous researchers. However, until now it is not known what the best dose is to support the improvement of the color quality of ornamental fish. This is the main focus so it is necessary to research to determine whether feeding formulations with different carotenoid sources effect on improving the color quality of Sunkist balloon molly fish (*Poecilia* sp.).

METHODOLOGY

Place and Time

This research was conducted from March 2022 to April 2022 at the Fisheries Cultivation Laboratory, Marine and Fisheries Education Study Program, Universitas Pendidikan Indonesia, Serang Regional Campus with a maintenance period of 35 days.

Research Materials

The test fish used in this study were Sunkist balloon molly fish (Poecilia sp.), female aged 4-5 months, obtained from ornamental fish collectors in Taman Sari, Serang City, Banten. The stocking density of the test fish was 5 fish/aquarium. The tools used in this study were 4 aquarium units measuring 60×30×30 cm. aquarium, digital scale, aerator, DSLR camera (Digital Single Lens Reflex), ImageJ 1.440 (Image Processing and analysis in Java) for image analysis digital color quality, DO meter, pH universal test paper, digital thermometer, millimeter block, drain, siphon hose, bucket, sieve or sieve, plastic basin, blender, grinder machine for making feed for feed formulation printing, plastic, waring. The feed used during the study consisted of factory-made feed (fengli 0), marigold flower flour (T. erecta L.), shrimp head flour, pumpkin flour (C. moschata D.), supplemented with fish meal, fine bran, tapioca flour as a binder, vitachick functions as an additional vitamin, ultramineral as a mineral ingredient in feed.

Research Design

This study used an experimental method with a completely randomized design (CRD) which included 4 treatments, each treatment was repeated 3 times consisting of a control treatment

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P₀ (feeding of pellets fengli 0), treatment P₁ (feeding of rice bran 12.87 % + fish meal 72.12% + marigold flower flour 10%), P₂ treatment (feeding rice bran 12.87% + fish meal 72.12% + shrimp head flour 10%), P₃ treatment (feeding rice bran 12.87% + fish meal 72.12% + pumpkin flour 10%). The experimental method was chosen in this study to obtain the results of the analysis in the form of primary data so that it can be proven concretely to researchers regarding their findings during the study.

Work Procedure

The aquarium has been cleaned and equipped with an aeration setting to 50 L of water volume and a net as a cover. A total of 5 healthy Sunkist balloon molly fish aged 4-5 months were acclimatized in each aquarium. Feed preparation was done by compiling the composition of the feed according to the needs of the fish (Table 1). Feed formulation is regulated by a protein content target of 40%. The process of making feed namely the selection of raw materials, mixing of raw materials, making pellets, drying, and storage. Fish were reared for 35 days and fed 5%/day of biomass weight. Siphoning 30% water/week to remove the waste. Fish sampled during the study included the number, weight, and body color of fish. The test sample was photographed in a flat aquarium using a DSLR camera without anesthesia. LED lights were used as a lighting source with a shooting distance of 10 cm (Kusumah et al., 2011). The images were saved in JPEG file format and analyzed with ImageJ 1.440 software to produce RGB (Red, Green, Blue) and HSB (Hue, Saturation, Brightness) colors, and then characterized and quantified to determine differences in color quality.

Table 1.Feed ingredients in each treatment.

	Treatment dose in feed (%)				
Feed Ingredients	P_1	P ₂	P ₃		
Fish flour	72.12	72.12	72.12		
Fine bran	12.87	12.87	12.87		
Marigold flour (Tagetes erecta L.)	10	0	0		
Shrimp head flour	0	0	10		
Pumpkin flour (Cucurbita moschata D.)	0	10	0		
Tapioca flour	1	1	1		
Vitamin	1	1	1		
Mineral	3	3	3		

Table 2.	Proximate co	omposition	of the test	feed in	research	(% wet	weight).
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		1						
-	Feed	Water Content	Ash	Drotoin	Linid	Carbohydrate		
	Treatments	Water Content	Content	Ploteill	гіріа	Fiber	BETN	
-	Po	11%	13%	40%	5%	2%	-	
	P_1	7.32%	12.09%	31.10%	7.25%	5.46%	36.78%	
	P ₂	7.83%	11.32%	29.92%	7.46%	6.11%	37.36%	
	P ₃	7.76%	12.00%	34.27%	8.03%	6.26%	31.68%	

Data Analysis

The research data were analyzed using statistical software Microsoft Excel and SPSS IBM 25. Data analysis was initiated by performing normality and homogeneity tests on the research data. Then, a statistical test of variance was carried out using the One Way ANOVA (Analysis of Variance) test with a 95% confidence level. If there is a significant difference (P < 0.05) between treatments, it is followed by a further test of Tukey HSD or BNJ (real difference honestly) at a 5% level, to determine the location of the significance of the data. Finally, descriptive analysis is used to measure quality by comparing research data and previous research data.

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RESULTS AND DISCUSSION Feed Utilization Efficiency

The efficiency of feed utilization is related to the addition of the biomass weight on the body of the Sunkist balloon molly fish (*Poecilia* sp.) utilization of protein in the feed given. Comparison results between the increase in body weight of fish with the amount of feed consumed during maintenance shows the value of feed utilization efficiency.



Figure 1. Average percentage of fish feed utilization efficiency Sunkist balloon molly fish (*Poecilia* sp.). Numbers with the same letter notation square mean no there is a significant difference between treatments.

Based on Figure 1, it is proven that the P_0 treatment (control feed), the marigold flower flour mixture in the P_1 treatment and the shrimp head flour mixture in the P₂ treatment resulted in a value below the optimum range for feed utilization efficiency, which is below 25%. Meanwhile, the ideal benchmark for feed utilization efficiency values > 25% ranges from 35% - 100% (Mustofa et al., 2018). The low efficiency value of using artificial feed is due to the low digestibility of feed, because the feed ingredients used are mostly sourced from plant materials compared to animal materials (Nurfitasari et al., 2020).

The results of the P_3 treatment (vellow pumpkin feed) showed an efficiency value of the highest feed utilization of 40% when compared to treatment on the other hand, it can be seen from the results of the one-way ANOVA test of variance for the P_3 (pumpkin feed treatment yellow) shows results that are significantly different or have an effect on efficiency feed utilization (P < 0.05) so that Tukey's further test is carried out HSD or BNJ (Honest Significant Difference), so it can be seen that Sunkist balloon molly fish (*Poecilia* sp.) in P_3 treatment were able to utilize feed properly because the

nutritional needs of fish, especially protein, are fulfilled with good, according to the habits and nature of the fish so that feeding is more efficient. This means that the quality of the formulated artificial feed is quite good at a high level.

Feed Conversion Ratio

The data entered is the results of the calculation of the amount of feed spent during later research divided by the result of the final fish weight plus the weight of dead fish then subtracted by the initial weight. Complete feed conversion ratio data can be seen in (Fig 2). Feed conversion ratio of Sunkist balloon molly fish (Poecilia sp.) showed the highest to lowest feed conversion ratio value in each treatment, namely P_0 (control feed) of 4.7, P₂ (shrimp head feed) of 3.86, P₃ (yellow pumpkin feed) of 3.69, P1 (feed of marigold flowers) of 3.61. Based on the results of the one-way ANOVA analysis of variance given feed formulations with different carotenoid sources to improve quality color in treatment P1 (marigold flower feed) showed different results significant or effect on feed conversion ratio (P < 0.05) so that a further tests of Tukey HSD or BNJ (Honest Significant

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Difference). The value of the feed conversion ratio is inversely proportional to the growth value weight and length growth, the higher the growth value, the lower feed conversion ratio value, this indicates that the depleted feed consumed by fish can be put to good use by the test fish through the process of metabolism so that it can support fish growth. Sourced on the results obtained in this study it can be seen that the feed conversion ratio of Sunkist balloon molly fish (*Poecilia* sp.) is in the fairly good category. Ratio value feed conversion ranged from 1.5 to 8, meaning the feed conversion value in all treatments can be said to be good because in general, it is still in the normal range.



Figure 2. Average feed conversion ratio Sunkist balloon molly fish (*Poecilia* sp.). Numbers with the same letter notation square mean no there is a significant difference between treatments.

Growth Performance

Table 3.

Growth performance included absolute weight growth, absolute length

growth and survival rate shown in Table 3 below.

	I I.a. i.e.	0	Result			
Variable	Unit	P_0	P_1	P_2	P_3	
Absolute Weight Growth	g	0.1	0.1	0.1	0.2	
Absolute Length Growth	cm	0.1	0.1	0.1	0.1	
Survival Rate	%	100	100	100	100	

Growth performance of fish during research.

Based on the results of data analysis absolute growth in fish is closely related to the availability and the ability of fish to absorb nutrient sources in rearing feed, where the Sunkist balloon molly fish (Poecilia sp.) has a digestive tract that is shorter than other fish because this fish is omnivorous facilitate the process of absorption of nutrients in the body. Complex compounds protein essential amino acids and non-essential are needed to support the growth process and also as a producer of energy sources (Iskandar and Fitriadi, 2017). Sourced on the results of the one-way ANOVA analysis of variance in the feeding formulation with different carotenoid sources to improve

color quality in P₃. treatment (yellow pumpkin feed) showed significantly different results or affected absolute weight growth (P<0.05) this is in line with the protein content contained in pumpkin feed obtained the value of protein content which is high when compared with other test feeds proves that the provision of carotenoid source feed formulations is not only utilized for improving the quality of his body color, on the other hand, is also used for absolute weight gain.

The minerals contained in fish feed can be used as a benchmark which affects the absolute length growth. Added minerals into the test feed about 3%

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contains calcium, phosphorus, and magnesium, and most of these minerals are found in skin waste and crustacean shells (Sukarman and Sholichah, 2011). Based on the results of the analysis one-ANOVA variance way of feeding formulations with carotenoid sources on the improvement of color quality in the P_2 treatment (shrimp head feed) show results that are significantly different or have an effect on growth absolute length (P < 0.05) this is in line with the selection of raw materials in the manufacture of feed formulations using shrimp head waste with a variety of high mineral content proven to be able to support the growth the absolute length of the Sunkist balloon molly fish (Poecilia sp.).

Based on observations of the survival rate Sunkist balloon molly fish (*Poecilia* sp.) for 35 days of maintenance shows the average survival rate of the test sample reaches 100% in all treatments, meaning that during the study the test sample did not experience death, this can be due to the adaptability of fish to such as feed sized feed according to mouth opening and artificial feed made from selected carotenoid sources have met the nutritional needs of the feed during the maintenance period of Sunkist balloon molly fish (*Poecilia* sp.) so that support metabolic performance, if the metabolic rate in the fish body is stable then the appetite increases and also the low mortality rate of fish is supported by the fish body immunity is maintained (Hidayat *et al.*, 2013).

Color Quality

Adduction feed formulations with different carotenoid sources can affect improving the color quality of Sunkist balloon molly fish (Poecilia sp.), characterized by an increase in the average RGB value (Red, Green, Blue) and HSB (Hue, Saturation, Brightness) which fluctuate on every treatment during maintenance, meaning that there is treatment in feeding source of carotenoids with the best results and not good for increasing color quality of Sunkist balloon molly fish (Poecilia sp.). The target of regarding color observation quality improvement is aimed at all parts of the Sunkist balloon molly fish (Poecilia sp.) including the head, abdomen and tail which were then analyzed using imageJ software (Image Processing and analysis in Java) 1.440 so that it can be seen the difference in the average value of increasing the color quality of the Sunkist balloon molly fish (Poecilia sp.) (Figure 3).



Figure 3. Average of color quality molly balloon Sunkist fish (Poecilia sp.).

Sunkist balloon molly fish (*Poecilia* sp.) is a type of omnivorous fish that has sufficient absorption capacity high in carotenoids with a polar structure contained in pumpkin such as astaxanthin (red), canthaxanthin (red), β -carotene (orange), lycopene at 7 g/Kg dry weight,

and also contains 175 g/Kg dry weight lutein which gives a bright yellow color, zeaxanthin (orange) (Madiara *et al.*, 2019). The process of absorption of carotenoids in the body starts from the stage where Sunkist balloon molly fish (*Poecilia* sp.) consumes feed containing

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sources of carotenoids that enter the body dissolved by fat and digested in the intestine assisted by pancreatic lipase and bile salts. These enzymes will hydrolyze carotenoids and then channel into the cytoplasm of mucosal cells small intestine and are broken down into retinol (a vitamin A derivative) into the blood vessels and transported to the liver (lymphatics) then deposited into the cells the chromatophores pigmented tissue contained in the dermis is placed accordingly types of colors such as xanthophore (yellow) and erythtrophore (red and orange) (Subamia *et al.*, 2010).

The absorption of carotenoids that are processed in the body's tissue cells will affect the pigment cells so that they give rise to color, in addition to the provision of carotenoids in the form of pumpkin flour as much as 10% with a protein content of 34.27%, crude fiber 6.26%, BETN 31.68%. It is suspected that the pigment absorption process has been sufficient to be synthesized so that the quality of the colors displayed is brighter.

Table	4.	Digital	values	of bo	ody (color	on	the H	ISB m	odel.	

Trootmonto	N	N Remark	HSB Ratio of Body			
Treatments	IN		Н	S	В	
Po	15	% Mean	23%	22%	23%	
P_1	15	% Mean	27%	23%	22%	
P ₂	15	% Mean	32%	26%	25%	
P ₃	15	% Mean	32%	27%	27%	

Based on the concept of digital value for RGB (Red, Green, Blue) color model of each component makes up more colors difficult to understand because each color depends on the result of mixing three color components (Red, Green, Blue), in other words, each of these components does not stand alone (free) while the HSB color model (Hue, Saturation, Brightness) shows the color space in the form of three main components. Therefore, only the HSB (Hue, Saturation, Brightness) color model is displayed in this research. The range, mean and percentage (%) are included in the results at the end of the characterization of each treatment. A color level that comes from the spectrum of light that is captured by the eye and includes a reflection of the structure and color of carotenoids known as hue (Junianto and Zuhdi, 2018). Hue value range, angle 0°-360°. The value 0° indicates red-orange, 60 indicates yellow, 120 is green, 180 cyan, 240° is blue and 300° is magenta. Saturation (saturation), is the level of color purity due to the influence of the basic color (white) just like red is influenced by white, a combination of red to pink with a range of 0 - 100 is worth percent (%). Brightness indicates the

value of brightness, generally measured as a percentage value (%) from 0% (black/white) to \pm 150% (red/orange).

Characterization of reddish orange color is determined by the type of color (hue) with a value of 11° - 37°, saturation point color 12% - 87%, and color brightness 14% - 68% (Kusumah et al., 2011). The findings on HSB (Hue, Saturation, Brightness) values in this study that the orange color redness is characterized by color type (hue) with a value of 23º - 32º, saturation point color 22% - 27%, and color brightness 22% -27% in detail can be found in (Table 4). Color each reddish orange is determined by the value of the brightness level of the color and color saturation while the value of color type (hue) is only quantified as a color characteristic (such as red, orange, orange) based on light reflected by objects or commonly called pure color (Junianto and Zuhdi, 2018).

The HSB color model (Hue, Saturation, Brightness) digital values of color on the whole body before further analysis is preceded by normality and homogeneity tests. The amount of data obtained includes small data (n<30), then the normality test used is the Shapiro-Wilk

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The one-way ANOVA (analysis of variance) variance test was continued. From the results of the one-way ANOVA (analysis of variance) variance test, it shows a significance or probability value of 0.041 or (P < 0.05) so that the treatment of feeding formulations with carotenoid sources is significantly different or has a significant effect on the color quality of Sunkist balloon molly fish

(Poecilia sp.). Further tests were carried out using the Tukey HSD test or BNJ test (honest significant difference) with a level of 5% proving that feeding formulations with different carotenoid sources affected the quality improvement the color becomes reddish-orange in the Sunkist balloon molly fish (Poecilia sp.), the P2 (shrimp head 10%) and P₁ (marigold flower 10%) test treatments were not significantly different from the P₀ (control) treatment. While the P₃ test treatment (10% pumpkin) was significantly different from the P₂ test treatment (shrimp head 10%) and the P_1 test treatment (10%) marigold flowers) and was verv significantly different from the P₀ (control) treatment so the provision of carotenoid sources in the form of pumpkin as much as 10% gave the best results for improving the color quality of Sunkist balloon molly fish (*Poecilia* sp.) with the highest average standard deviation of 202.67 \pm 27.2 among other treatments (Figure 4).





The complete visual appearance of increasing color quality of Sunkist balloon molly fish (*Poecilia* sp.) which is

characterized by changes in color quality, can be seen in Table 5.

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Treatment	Week-0	Week-4
P ₀ (Fengli 0)		
	P ₀ (A)	P ₀ (A)
P ₁ (Marigold Flower Feed)	000	
	P ₁ (B)	P ₁ (B)
P ₂ (Shrimp Head Feed)		
	P ₂ (A)	P ₂ (A)
P ₃ (Pumpkin feed)		100
	$P_{2}(A)$	$P_{2}(A)$

 Table 5.
 Comparison of color quality of Sunkist balloon molly fish (*Poecilia* sp.) at the beginning and end of maintenance.

Water Quality

Observations of water quality carried out in this study were used as supporting data, where all the environmental factors of the water are attempted to provide optimal results for the maintenance of Sunkist balloon molly fish (Poecilia sp.). Water quality measurement parameters in this study include temperature, pH, and dissolved oxygen (DO). The value of each water quality parameter that observed trend to be normal and stable, from the beginning to the end of the maintenance period because the feed given does not harm water quality (Addini et al., 2017). Temperature measured using a digital thermometer during the maintenance period Sunkist balloon molly fish (Poecilia sp.) averaged 28.5-28.7 °C in each treatment. The next water quality parameter is pH (degree of acidity) measurements that have been carried out universal test paper on using all treatments produced a value that has exceeded the water quality threshold, with

the average pH value obtained between 6.0 with the value obtained indicating the condition of the pH of the water that is not variable, tends to be stable from the first measurement to measurement final. Then, the range of the average value of the content dissolved oxygen (DO) as measured using a portable DO meter is at the optimal range for ornamental fish cultivation activities is > 3 mg/L between 5.6 - 5.7 mg/L (Solihah *et al.*, 2019).

CONCLUSION

Adduction feed formulation with the addition of carotenoid sources in the form of pumpkin (*Cucurbita moschata D.*) in P₃ treatment had a significant effect on P₂ treatment (shrimp head) and P₁ treatment (marigold flowers) and had a very significant effect on P₀ treatment (control) so that the provision of feed formulations with carotenoids in the form of pumpkin as much as 10% gave the best results on improving the color quality of Sunkist balloon molly fish (*Poecilia* sp.) giving a reddish orange color change. Feed

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utilization efficiency, feed conversion ratio and growth performance were not significantly different between treatments.

ACKNOWLEDGEMENT

The authors thank all parties who have aided in the completion of this research.

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