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Effect of supplemented liquid pigments in the diet on growth performance and body skin color of Jewel cichlid (*Hemichromis lifalili*) fish

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Abstract The weight, average daily gain (ADG), and body skin color values of Jewel cichlid (*Hemichromis lifalili*) fish were measured on the 0, 30, 60, and 75 days of the experiment. The fish received liquid feed for the first 60 days and no supplementary feed from day 61 to 75. The body weight and ADG of fish raised for 0-30, 0-60, and 0-75 days was not statistically significant differences ($p<0.05$). However, it was found that fish fed with the supplemented different pigments had different L* and a* values at each stage of the experiment, i.e. when fish were fed with diets containing natural capsanthin and synthetic astaxanthin for 60 days, the L^* and a* color values were higher than those of the other groups. The L*, and a* values were lowered after the liquid pigments supplementation was withdrawn.

Keywords: Astaxanthin, Canthaxanthin, Capsanthin, Fish body skin color, Ornamental fish

Introduction

The fish in Cichlidae family includes the Jewel cichlid (*Hemichromis lifalili*), a freshwater fish native to parts of South Africa and Central Africa. The Jewel cichlid is renowned for its vivid colors and striking patterns. It is hardy and easy to breed, making it one of the most popular ornamental fish species in both local and international markets. Fish must obtain their pigment from feed because they are unable to produce much of it on their own in the wild. To enhance coloration, ornamental fish breeders often incorporate both artificial and natural colorants into fish feed, allowing pigments to accumulate in different parts of the body. Pigmentation is also one of the important quality attributes of the aquatic animal for beautiful fish lovers' acceptability.

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Carotenoids are the most widespread pigments found in nature, occurring in photosynthetic bacteria, fungi, algae, plants and animals (Maoka, 2020). These pigments are responsible for the coloration of the muscles and skin of ornamental fish and the exoskeleton of shrimp (Shah *et al.*, 2013). Carotenoids are important for the rearing of fish and other aquatic animals as they form the basis for pigmentation in various species, tissues and ecological parameters. Both synthetic and natural carotenoids can induce pigmentation in aquatic animals. Natural carotenoids include lutein, zeaxanthin, capsanthin and astaxanthin, which contribute to the yellow, orange and red colors of the skin. Lutein and zeaxanthin enhance yellow and orange pigments, while capsanthin, astaxanthin and canthaxanthin enhance red pigments.

Carotenoids have several functions, including pigmentation, antioxidant activity, source of vitamin A, improved growth, improved survival, stress resistance and better reproductive potential (Britton, 2008). Currently, synthetic astaxanthin is the main carotenoid pigment used for the reddish-pink coloration of aquatic animals. However, the high cost and environmental impact of synthetic pigments have led to concerns about their use. Studies show that natural pigments have a higher bioavailability than synthetic ones. For example, red paprika, which contains natural capsanthin, provides an orange-red coloration to the skin and muscles of animals. Red paprika or red carotenoids also contains zeaxanthin, beta-carotene, lutein, capsorubin and capsanthin. The addition of liquid pigments is a practical method of improving the coloration of animal feed.

Therefore, this study aimed to evaluate the effects of liquid pigments on the growth performance and coloration of Jewel cichlid by supplementing liquid natural capsanthin, synthetic astaxanthin, synthetic canthaxanthin, and natural capsanthin blended with synthetic astaxanthin and synthetic canthaxanthin to the diet comparable with non-supplemented liquid pigments.

Materials and methods

Fish feed and feeding

The experimental fish feed contained 42% protein. It contained anti-stress balance (AV3®, Manghebati SAS, France), then coated with a water-based feed coating (chitosan) to make the feed durable and protect the insoluble color leaked out. To prepare the liquid pigment mixture, each liquid pigment was measured and mixed with 100 µl of anti-stress balance before being incorporated into 100 grams of feed. Subsequently, 2,000 µl of chitosan was sprayed onto the feed. In the experiment, five different types of liquid pigments were added to 100 grams of fish feed, with the amounts used listed in Table 1. For each treatment, 4,000 µl of water was mixed with the respective liquid pigment per 100 grams of feed.

There were 5 treatments as follows: control treatment without liquid supplementation, supplemented with liquid synthetic astaxanthin, supplemented with liquid synthetic canthaxanthin, supplemented with natural capsanthin, and supplemented with mixed synthetic astaxanthin, synthetic canthaxanthin, and natural capsanthin.

Table 1. The table shows the color combinations used in each test group

treatment	AV3[1/]	chitosan	water	astaxanthin	canthaxanthin	capsanthin
1	100 µl	2,000 µl	4,000 µl	-	-	-
2	100 µl	2,000 µl	2,500 µl	1,500 µl	-	-
3	100 µl	2,000 µl	2,500 µl	-	1,500 µl	-
4	100 µl	2,000 µl	250 µl	-	-	3,750 µl
5	100 µl	2,000 µl	1,750 µl	500 µl	500 µl	1,250 µl

[1/] AV3®, Manghebati SAS, France

Each 100-gram batch of feed was thoroughly mixed to ensure an even distribution of pigments. This mixing process was repeated every three days to produce a new batch of feed, as older feed was considered unsuitable for use.

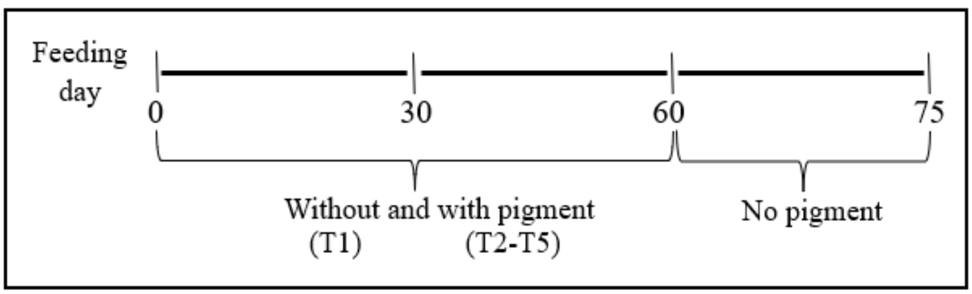

Figure 1. The feeding schedule for each experimental group

Experimental design and rearing procedure

The experimental design was completely randomized design with 5 treatments and there were 3 replications in each treatment. There were 15 water tanks with the dimension of 20x12x15 inches. The experiment was carried out in 15 tanks with each of 57.6L capacity that contain 46.0L of water. Three-month-old Jewel cichlids with an initial weight between 3.20-3.50 grams were introduced at a density of seven fish per tank. The fish were randomly allocated to the individual tanks and the feed treatments were also randomly assigned to the tanks. The fish were fed the experimental diet twice daily, at 8:00 am and 4:00 pm, until satiation. To maintain the water quality, 50-70% of the water and feed waste was siphoned off every two days and replaced with fresh water. All water parameter was controlled in optimum of fish culture, DO>5.5 mg/L, and pH 7.4-8.3. The trial period was 75 days.

Data collection

The fish weight, average daily gain, and skin color were measured on 0, 30, 60, and 75 days. To measure fish body skin color, MiniScan EZ45/0 LAV, illuminance D65, Hunter Associates Laboratory Inc., Reston, VA, USA) was used to measure L* (lightness), a* (redness), and b* (yellowness) color values. Three fish in each water tank were randomly selected to measure body skin color. Skin color was measured under anesthesia (1-2 ml/L of clove oil) from fish in each tank. Each fish was measured each color came from an average of 3 times measuring and regions close to the dorsal section (midway between the head and the tail).

Statistical analysis

Descriptive statistics analysis, mean and standard deviation were used to summarize the weight, average daily gain (ADG), and body skin color (L*, a*, and b*) at the 0, 30, 60, and 75 days of the experiment.

Inferential statistics analysis was one-way analysis of variance was used to compare the differences in weight and ADG at the 0, 30, 60, and 75 days of fish fed with different 5 types of liquid pigments, and one-way repeated measure analysis of variance was used to compare the differences in body skin color (L*, a*, and b*) of fish fed with different 5 types of liquid pigments at the 0, 30, 60, and 75 days of the experiment. The structural models of each inferential statistics were one-way analysis of variance. The experiment was a completely randomized design with 5 treatments and 3 replicates or tanks per treatment. Each fish tank with 5 fish was considered as the experimental unit for investigating the fish's weight and ADG which measured at the times of 0, 30, 60, or 75 days. The equation of the statistical model was $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} was the observed dependent variable (weight and ADG), μ was the overall mean, T_i was the effect of treatments, and e_{ij} was the random error.

one-way repeated measure analysis of variance: The experiment was a completely randomized design with 5 treatments and 3 replicates or tanks per treatment. Each fish tank with 5 fish was considered as the experimental unit for investigating the fish's body skin color (L*, a*, and b*) which was measured at the times of 0, 30, 60, or 75 days. The equation of the statistical model was $Y_{ijk} = \mu + T_i + Time_j + T_i * Time_j + e_{ij}$, where Y_{ij} was the observed dependent variable (body skin color (L*, a*, and b*), μ was the overall mean, T_i was the effect of treatments, $Time_j$ was the measuring days, $T_i * Time_j$ was the interaction between treatments and times, and e_{ij} was the random error.

All data were analyzed using SPSS version 17.0. The Duncan test was used to determine differences among treatment means at the confidence level of 95% (p=0.05).

Results

The results of supplementation with 5 different liquid pigments in Jewel cichlid on weight and ADG are shown in Tables 2 and 3. Supplementation with 5 different types of liquid pigment found that the fish's body weights and ADG measured on days 0, 30, 60, and 75 days of the experiment were not statistically different (p>0.05).

Table 2. Effect of various liquid pigment supplements on the body weight (g) of Jewel cichlids at different durations of feeding days

Treatment	Days After Supplementation			
	0	30	60	75
Control	3.41±0.03	5.91±0.06	7.42±0.65	7.58±0.13
Astaxanthin	3.45±0.03	5.21±0.41	6.89±0.27	6.71±0.31
Canthaxanthin	3.40±0.00	5.31±0.22	7.13±0.40	7.13±0.32
Capsanthin	3.37±0.06	5.61±0.09	7.30±1.73	6.93±1.11
Mix[1/]	3.44±0.03	5.64±0.21	6.60±0.19	6.63±0.43
p-value	0.148	0.552	0.784	0.322

[1/] Feed containing a liquid mixture of astaxanthin, canthaxanthin, and capsanthin

Table 3. Effect of different liquid pigment supplements on average daily gain (g/d) of Jewel cichlid fish across different rearing periods

Treatment	Rearing period		
	0-30 day	0-60 day	0-75 day
Control	0.08±0.02	0.06±0.01	0.05±0.00
Astaxanthin	0.05±0.01	0.05±0.00	0.04±0.00
Canthaxanthin	0.06±0.00	0.06±0.01	0.05±0.00
Capsanthin	0.07±0.03	0.06±0.03	0.04±0.01
Mix[1/]	0.07±0.01	0.05±0.00	0.04±0.00
p-value	0.540	0.803	0.485

[1/] Feed containing a liquid mixture of astaxanthin, canthaxanthin, and capsanthin

The measurements of body skin color (L* for lightness, a* for redness and b* for yellowness) of Jewel cichlids supplemented with five different liquid pigments over four measurement days is shown in Table 4. The analysis was not statistically significant interacted between treatment and measurement time for any color parameter (p>0.05). However, the different pigments had significantly affected the L* and a* values (p<0.05), but not on the b* values (p=0.240). Different time points significantly influenced the L*, a* and b* values (p<0.01).

Fish fed with mixed pigments had the lowest L* value (34.47), indicating darker skin. Fish fed with natural capsanthin and synthetic astaxanthin had the highest a* values (10.12 and 8.96, respectively), indicating greater redness. The skin color values L*, a* and b* on the different days of feeding were statistically significantly different (p<0.05).

The L*, a* and b* values of fish fed with pigments for 60 days were higher than those of fish fed for 0 and 30 days. After withdrawal of pigments for 15 days after the 60-day feeding, L*, a* and b* levels decreased significantly ($p<0.05$).

Table 4. Estimated marginal means (EMM) and standard error (SE) of 5 different liquid pigment supplements and 4 measuring times of Jewel cichlid fish's colors (L*, a*, and b*) and *p*-values of testing the differences between liquid supplement, time, and interaction between liquid supplement and time

Factors	**EMM±SE**		
	L*	**a***	**b***
Pigments			
Control	39.17^{a}±0.85	5.04^{b}±0.96	7.94±1.25
Astaxanthin	37.34^{ab}±0.85	8.96^{a}±0.96	9.82±1.25
Canthaxanthin	38.10^{ab}±0.85	4.73^{b}±0.96	8.77±1.25
Capsanthin	35.94^{bc}±0.85	10.12^{a}±0.96	11.88±1.25
Mix[1/]	34.47^{c}±0.85	6.98^{ab}±0.96	8.17±1.25
	EMM±SE		
Time			
0 day	38.02^{x}±0.68	3.68^{z}±0.28	8.59^{x}±0.44
30 days	35.72^{y}±0.79	7.29^{x}±0.67	9.25^{x}±1.06
60 days	41.58^{w}±0.57	10.98^{w}±1.11	13.36^{w}±1.21
75 days	32.70^{z}±0.83	6.70^{y}±0.56	6.07^{y}±0.63
***p*-value**			
Pigment	0.023	0.010	0.240
Time	0.000	0.000	0.002
pigment*Time	0.260	0.088	0.558

[1/] Feed containing a liquid mixture of astaxanthin, canthaxanthin, and capsanthin

[a, b, c, d, / w, x, y, z] different letters in the same column means the averages are statistically significantly different ($p < 0.05$)

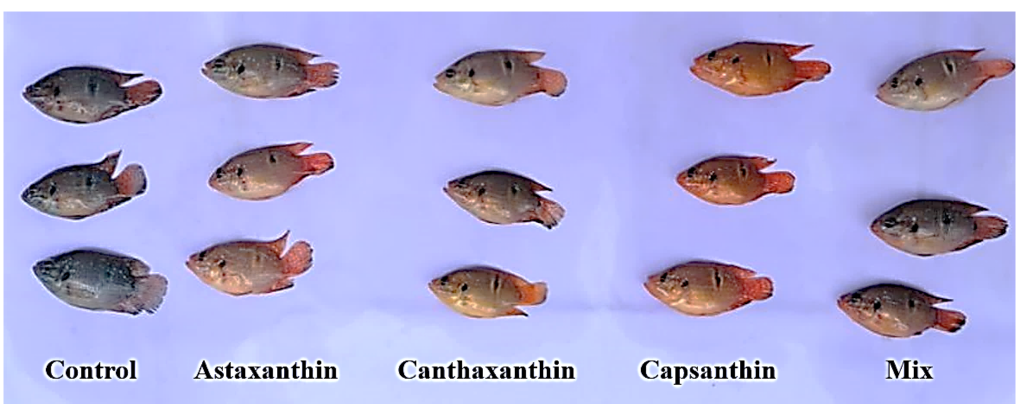

Figure 2. Jewel cichlid fish images are shown in each group

Discussion

Measurements of body weight and ADG between 0-30 days, 0-60 days and 60-75 days in Jewel cichlids fed five different liquid pigments for 60 days, followed by a 15-day break in supplementation, showed no statistically significant differences. These results indicate that pigment supplementation had no effect on the growth performance of Jewel cichlids over 30, 60 or 75 days. These results are consistent with a previous study (Harpaz and Padowicz, 2007), which found no significant effects of carotenoid supplementation with paprika oleoresin on growth rate and survival of dwarf cichlids ($p>0.05$). Another study (Kop *et al*., 2010) also found that the addition of red pepper to the diet did not result in differences in feed conversion and growth of cichlids (*Cichlasoma severum*), as weight and ADG did not differ significantly in all groups. It has been reported that the carotenoids may improve nutrient utilization. For example, rainbow trout supplemented with carotenoids showed higher growth performance compared to those without supplementation (Amar *et al*., 2002; Mirzaee *et al*., 2013).

However, feeding Jewel cichlids with five different liquid pigments in their diet affected their body skin color in terms of L* (lightness), a* (redness) and b* (yellowness). The group fed with mixed pigments showed the lowest L* value, while the group fed with natural capsanthin and synthetic astaxanthin showed the highest a* values. Astaxanthin is known to be more readily absorbed and accumulated in fish compared to other carotenoids (Torrisen *et al*., 1989). In this study, the mixed pigment group contained synthetic astaxanthin, synthetic canthaxanthin and natural capsanthin, suggesting that astaxanthin and capsanthin contributed significantly to the observed increase in a* values ($p<0.05$).

It is plausible that capsanthin can convert to astaxanthin and accumulate in fish tissue. Previous studies (Hata and Hata, 1972, cited by Mirzaee *et al*., 2013) have shown that Jewel cichlids are able to accumulate various carotenoids, particularly in the form of astaxanthin, which contributes to the total carotenoid content in their skin.

The values for body skin color L*, a* and b* differed significantly between the different days of liquid pigment supplementation. In particular, fish fed pigments for 60 days had higher L*, a* and b* color values than those fed for 0 and 30 days. However, after 15 days of pigment deprivation following the 60-day supplementation, body skin color values of L*, a* and b* decreased.

This decrease indicates that pigment supplementation in the diet was discontinued during the withdrawal period, leading to a possible decrease in pigment accumulation in the fish's body.

In conclusion, the results of this study showed that the liquid pigments did not affect the Jewel cichlid fish's growth performance. However, the supplemented with liquid synthetic astaxanthin, natural capsanthin, or mixed pigments for 30-60 days had higher L* and a* values. These pigments are known to accumulate better in the skin of fish compared to other pigments and affect the hues associated with color-enhancing feed, especially dark red color.

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