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Influence of spirulina powder at carotenoids concentration in fin of an ornamental fish *Trichogaster lalius*

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Abstract
In the present study juveniles of *Trichogaster lalius* were exposed to different concentration of spirulina powder incorporated diets to observe the impact in fin pigmentation of the respective species in captive condition. Three different concentration feed were prepared by incorporating spirulina powder at the rate of 2, 4 and 6 g kg^{-1} and designated as A-2, A-4 and A-6 with one kept as control. Eight set of glass aquarium of 50l capacity (30 × 30 × 30 cm) were used for 4 set of experiment with their respective replicates. The impact of spirulina treated feed on fin pigmentation was analyzed for a period of 60 days. The variation in carotenoid level of fin was observed at wavelength of 450, 475 and 500 nm. Maximum carotenoid accumulation was observed in fin of those specimens fed with 6g kg^{-1}spirulina powder incorporated diet for 450, 475 as well as 500 nm wavelength respectively. There was significant increased (p<0.05) in carotenoid concentration in all three treatments at different wavelengths compared to control. The present findings established that 6 gkg^{-1}spirulina powder incorporated feed has very good potential to enhance the pigmentation of fin in the fish species, *Trichogaster lalius*.

Keywords: Spirulina powder, carotenoid concentration, pigmentation, *Trichogaster lalius.*

1. Introduction
The dwarf gourami, *Trichogaster lalius* (Hamilton, 1822) locally known as "lalia or kholse" an indigenous ornamental fish under Perciformes belongs to the family Osphronemidae is a peaceful ornamental fish and very popular to the aquarium hobbyists. Like other animals, fish cannot synthesize carotenoids in their body, and fishes must obtain them via food [1]. Therefore, carotenoid supplementation is needed to enhance the pigmentation in ornamental fishes. Ornamental fishes are mainly characterized by a wide diversity of colours, colour patterns and success of ornamental fish trade is very much dependent on the bouncy colour of the fish. Color is one of the major factors, which determines the price of aquarium fish in the world market [2]. Pigmentation in the skin is responsible for coloration of fish. The carotenoids act as a vital nutrient for healthy growth, metabolism, and reproduction as well as colour [3]. It is used in aquaculture feed to provide the colour associated with consumer product, such as the bright vibrant colours of ornamental fish [4]. Carotenoids are the primary source of the pigmentation on the skin of fishes. In natural environment, the fishes meet their carotenoid requirements by ingesting aquatic plants or through their food chains. More than 600 kinds of carotenoid are found in the nature, but few of them are used in animal food, medications, colour of food, polish chemicals [5, 6]. The colour enhancing diets should contain additional natural pigment to enhance the colour of the ornamental fishes. The freshwater ornamental fish industry has experienced the problem of faded coloration in fish, especially when the fishes are kept under captivity for long duration and also in intensive culture condition [7]. Enhancement of colouration is not only confined in ornamental fish industry, several scientists are engaged in improvement of colouration of muscle or skin of food fish as it is an important factor which determines the price of that fish in market. In spite of proper size, if the colour of the fish is not up to the mark, then the fish will not get a good market price. If enhancement of coloration can be done by administrating pigment enriched feed, it will definitely improve the quality and cost of the fish. Recent efforts have focused on natural compounds as alternative to synthetic carotenoids as because of concerns about the use of synthetic additives and their high cost. To alleviate this problem the present study was made to evaluate pigmentation quality using the

natural carotenoids. However, detailed studies on color enrichment through spirulina powder in ornamental fishes are lacking [8].

In this aspect, the present study was conducted to evaluate the effect of spirulina powder at carotenoids concentration particularly in fin of an ornamental fish *Trichogaster lalius* in control condition.

2. Materials and Methods

2.1 Experiment site

The experiment was carried out in the Department of Fisheries Resource Management, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal.

2.2 Collection and acclimatization of fishes

Juveniles' males of Dwarf gourami, *Trichogaster lalius* of uniform size group was purchased from local ornamental fish markets in the month of March, 2017. Fishes were transported to the laboratory by plastic bag with oxygen packing. In the laboratory the fishes were given a short bath treatment with 3 ppm $KMnO_4$ solution for 3 to 5 minutes for disinfection and subsequently, they were transferred to the rectangular tanks containing chlorine free tap water for acclimatization. Fishes were fed with prepared pellet feed (without spirulina) in two equal rations *i.e.* at 09.00 and 18.00 hours at the rate of 2% of their body weight.

2.3 Preparation of experimental design

After proper acclimatization healthy specimen were stocked into the experimental tanks for feeding experiment. A total 8 set of glass aquarium of 50 l capacity (30 × 30 × 30 cm) were used for 3 set of experiment with replicate and one set kept as control. In each tank 10 numbers of juveniles' were reared and the total experiment was conducted for 60 days. In the experimental tank fishes were first kept for a week with normal feed to make them adjusted with the environment. Suitable aeration and floating aquatic weed Hydrilla (*Hydrilla verticillata*) were provided to the tank to give the fishes a natural habitat.

2.4 Formulation of diet

Spirulina powder was mixed with the prepared pelleted feed at the rate of 2 g kg^{-1} (A-2), 4 g kg^{-1}(A-4), 6 g kg^{-1} (A-6) feed and one remained as control. The feed ingredients used to prepare the experimental diet provided in Table 1. Fishes were fed with experimental and control feed twice daily (09.00 and 18.00 hours) at 2% of body weight. Water was exchanged in each alternative day and left over feed and excreta were siphoned out every day.

Table 1: Proportion of feed ingredients used in formulated diet.

Name of ingredients	Percentage content in 100 gm feed
Fish meal	25
Soyabean meal	22
Groundnut oil cake	15
Rice bran	20
Wheat flour	12
Starch	3
Soya powder oil	2
Vitamin & mineral mix	1

3.5 Observation of carotenoid content

For estimation of total carotenoid content in the fin of tested fish, procedure was followed as described by [9] with following equation: 4 × Optical density value × total volume of sample taken / weight of sample (mg). Here the overall variation in carotenoid level of fin was observed at different spectro-photometric wavelengths (WL) *i.e.* 450 nm, 475 nm and 500 nm wavelengths.

3.6 Statistical analysis

The persistence rate of carotenoids in fin of dwarf gourami were statistically analysed through one way ANOVA and Paired t-Test by using the software packages (SPSS Statistics v16 and Microsoft Excel 2010).

4. Results and Discussion

4.1 Carotenoid concentration observed in fin at different wavelength

4.1.1 Observed at 450 nm wavelength

The total variation in carotenoid level of fin was observed at 450 nm is presented in Fig. 1. It was found that the initial carotenoid concentration in fin was 0.04 µg g^{-1} but at the end of the experiment the level was found 1.5 µg g^{-1}. Maximum carotenoid accumulation was observed in fin of those fish which fed upon 6 g kg^{-1} spirulina powder incorporated diet (6-A) followed by 2 g kg^{-1} (2-A) and 4 g kg^{-1} (4-A) spirulina powder incorporated diet. A gradual degradation in carotenoid concentration of fin was observed in fish which were not provided spirulina powder in their diet.

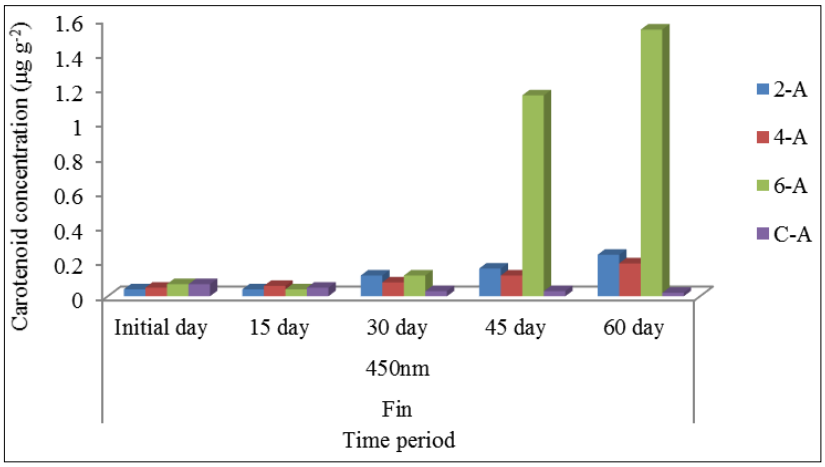

Fig 1: Carotenoid concentration in fin of *T. lalius* treated with different concentrations of spirulina power incorporated diet at 450 nm wavelength

4.1.2 Observed at 475 nm wavelength

Total carotenoid concentration in fin of *T. lalius* was also observed in 475 nm wavelength as shown in Fig.2. The results indicated that all three concentration of spirulina powder incorporated diet increased total carotenoid concentration in fin. The initial carotenoid value in fin was 0.04 µg g^{-1} but on 60th day of sampling the carotenoid value went up to 0.5 µg g^{-1}. The maximum carotenoid accumulation was observed in those fishes which were provided 6gkg^{-1} spirulina powder incorporated diet.

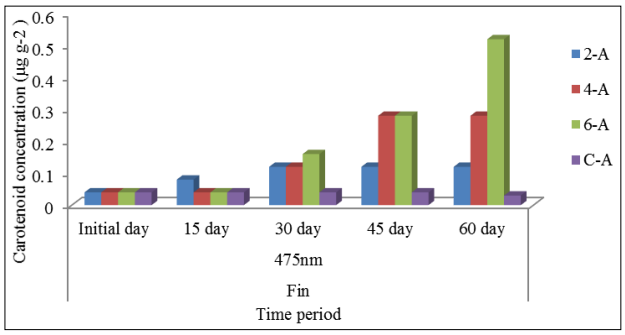

Fig 2: Carotenoid concentration in fin of *T. lalius* treated with different concentrations of spirulina power incorporated diet at 475 nm wavelength

4.1.3 Observed at 500 nm wavelength

The overall variation in carotenoid level of fin was observed at 500 nm presented in Fig. 3. It ranged from 0.04 to 0.158 µg g^{-1}. A maximum amount of carotenoid was recorded in 6 g kg^{-1} and 2g kg^{-1} spirulina powder incorporating diet fed fish and minimum level of carotenoids was seen in 4 gkg^{-1} spirulina and control diet fed fish.

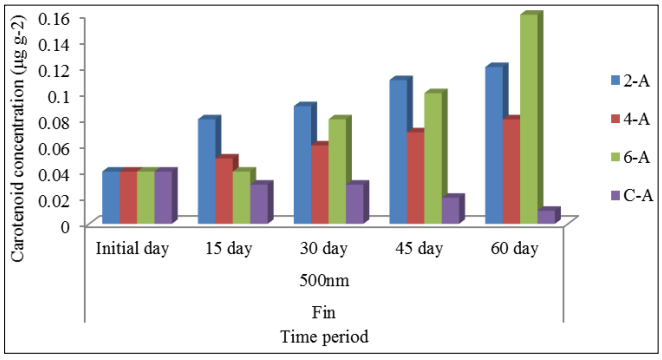

Fig 3: Carotenoid concentration in fin of *T. lalius* treated with different concentrations of spirulina power incorporated diet at 500 nm wavelength

The impact of different doses of spirulina powder in diet on fin colouration was statistically calculated by using statistical methods. The result obtained from t-Test showed that only two concentration 2 g $kg^{-1}$and 6 g kg^{-1} incorporated feed increased the fin carotenoid concentration significantly (p<0.05) highest at 450 and 475 nm wavelength respectively compare to other treatment and control. But at wavelength of 500 nm, it was found that 6 g kg^{-2} and 2 g kg^{-1} followed by 4 g kg^{-1} spirulina powder incorporated diet responded maximum carotenoid accumulation in fin compared to control. At 450 nm wavelength it was observed that supplementation of spirulina powder pigmented diet caused more carotenoid concentration in fin as well as the variability in colour of *T. lalius*. Spirulina incorporated diet at 6 g kg^{-1} caused a significant increase in carotenoid level of fin. A similar result was obtained by [10] in *Oreochromis niloticus* fed with spirulina powder. The carotenoid concentration in fin of *T. lalius* measured at 475 nm was increased with the increasing concentration of spirulina powder. But [11] found that addition of spirulina powder, paprika powder, Astaxanthin and Canthaxanthin did not show any improvement in carotenoid level of red and white coloured fancy carp *Cyprinus carpio* var. koi. So it can be mentioned that uptake of carotenoids from diet and the impact of carotenoids was varied from species to species. The result obtained at 500 nm described that with increasing dose of spirulina powder in diet, caused more carotenoid accumulation in fin of *T. lalius*. In case of fin the obtained results revealed that the fish fed with 6g kg^{-1} spirulina powder incorporated diet showed more increment in carotenoid level of fin as compare to other concentration of spirulina powder in diet. In case of Rainbow trout higher dose that was 10% of spirulina in diet resulted highest carotenoid deposition [12, 13]. Also found that 2.0% of beet root juice caused adequate colouration in both skin and fin of Rainbow trout. So it can be mentioned that a particular species response to a definite concentration of carotenoid source in diet which can vary from species to species or within the species [14]. Also noted that with increasing concentration of spirulina in diet in red swordtail, *Xiphophorus helleri*, the carotenoid accumulation in fin was also increased [15]. Reported same increasing pattern in fin carotenoid level of blue gourami, *Trichogaster trichopterus*. Spirulina diet of 6g kg^{-1} showed more accumulation of carotenoids in fin rather than any other concentration. It might be due to diet fed to fish at 6gkg^{-1} spirulina containing; maximum carotenoids absorbed from spirulina were transported and deposited into fin rather than any other location [17]. After addition of spirulina powder to diet of *T. lalius*, a significant increase in carotenoid concentration of fin was observed. Same result was found in case of rainbow trout by [12] when different concentration of *Spirulina platensis* meal provided to the fish. The overall carotenoid concentration in fin was showed an increasing trend from beginning to end of the experiment after adding spirulina to the diet. A similar kind of result was observed by [17, 18] in fin of gold fish and guppy *Poecilia reticulates* respectively.[15]reported that skin colour scores and total carotenoid concentration were significantly higher when blue gourami, *Trichogaster trichopterus* Pallas was fed spirulina algae supplemented diets for a period of 8 weeks followed by 4 weeks regular feed. They found that4g spirulina /kg feed give the highest result compare to 0, 1, 2 ad 3g spirulina /kg feed with no effect on survival rate.[14]observed that red swordtail, *Xiphophorus helleri* fed with 8% Spirulina incorporated diets increased the carotenoid concentration in fins, skin and muscle compare to 0, 1, 3 and 5%.[19]reported that addition of 60 mg oleoresin paprika per kg diet is sufficient to obtained good colouration in the ornamental dwarf cichlid *Microgeophagus ramirezi* [20]. Studied effects of spirulina powder in colour enhancement of *Trichogaster lalius* and found that 2 gkg^{-1} spirulina powder incorporated diets can enhance the fin pigmentation compare to other concentrations and control at 380 nm wave length. The results of the present study also with the agreement of other studies on enhancement of pigmentation while spirulina was added in supplemented diets and applied to the fishes, rainbow trout [21]; Crucian carp [22]; swordtails *Xiphophorus hellerii* [23] and red swordtails *Xiphophorus hellerii* [14].

The result of the present experiment suggested that dietary inclusion of 6gkg^{-1} spirulina powder incorporated diet was responsible for increased in fin carotenoid concentration

significantly (P<0.05) at 450,475 and 500 nm wave length respectively. The present finding established that spirulina powder incorporated diet is able to improve body pigmentation of dwarf gourami more effectively compare to the normal one.

5. Conclusion

In ornamental fish, colouration of skin and fin is very much important than that of muscle. The ornamental fishes are very popular due to its colouration and its persistence in the house aquariums. The dwarf gourami might fed with spirulina containing feed regularly while kept in control condition for enhancement and maintained its colouration. The advantage of spirulina powder is that, it's easily available in local market and can be smoothly incorporate with feed ingredients. Study revealed that, spirulina powder not only useful in enhancement of body pigmentation in fishes but also supports of growth efficiency in fishes. It can be concluded that a level of $6gkg^{-1}$ of spirulina powder in diet is ideal for persistence of colouration in the aquarium fish dwarf gourami in control condition.

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