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Spirulina powder as a source of carotenoid on pigmentation in the skin of dwarf gourami, *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 pigmentation of the respective fish in the captive condition. Three different concentration feed were prepared by incorporating spirulina powder at the rate of 2 g/kg (A-2), 4 g/kg (A-4) and 6 g/kg (A-6) feed and one kept as control. Eight set of glass aquarium of 50 l capacity (30 × 30 × 30 cm) were used for 4 set of experiment with their respective replicates. The impact of spirulina treated feed on skin pigmentation of fishes was analyzed for a period of 60 days. The overall variation in carotenoid level of skin was observed at different wave length *i.e.* 450 nm, 475 nm and 500 nm. At 450 nm wave length maximum carotenoid accumulation was observed in skin of those fish fed on 2g/kg followed by 4g/kg, 6g/kg spirulina powder incorporated diet and control. At 475 nm wave length the maximum carotenoid accumulation was observed on fish feed 6g/kg spirulina powder incorporated diet, and at 500 nm wave length maximum amount of carotenoid was recorded in 2g/kg followed by 6g/kg, 4g/kg spirulina powder incorporating diet and control. There was significant increased (P<0.05) in carotenoid concentration in all three treatments at different wave lengths compare to control. The present findings established that spirulina powder incorporated feed has very good potential to enhance the pigmentation of *Trichogaster lalius*.

Keywords: Spirulina powder, carotenoid, pigmentation, wave length, *Trichogaster lalius*

1. Introduction
Ornamental fish sector plays a vital role as a component of international fish trade, fisheries, aquaculture and development (FAO. 2014) [10]. Fish skin pigmentation, fin shape and size of the fish are the major factors which determine the market value of the ornamental fishes (Paripatananon *et al.* 1999) [20]. Carotenoids are a group soluble lipid pigments that are responsible for the red, orange and yellow colour in the skin, flesh, shell and exoskeleton of aquatic animal (Pailan *et al.* 2012) [19]. Dietary carotenoids play an important role in the regulation of skin and muscle colour in fishes (Ahilan *et al.* 2008) [1].
As fish are unable to convert intermediary precursor pigments to carotenoids (Liang *et al.* 2012) [16], pigments need to be supplied in the diet to achieve color formation. Due to the increasing cost of synthetic pigments and the public concerns on the use of synthetic additives, many alternative natural carotenoid sources have also been used (Jagadeesh *et al.* 2015) [13]. The fish *Trichogaster lalius*, mainly the male is having high domestic as well as foreign demands to the aquarium traders due to its sparkling, translucent blue colour with red or dark orange stripes in the body. For this reason, dwarf gourami (*Trichogaster lalius*) prefers for the colour enhancement treatment due to increase its demand in local as well as global market. Spirulina powder, easily available in local market and can be smoothly incorporate as feed ingredients for enhancing the body colour of any ornamental fishes. In this aspect, the present study was conducted to evaluate the effect spirulina powder at carotenoids concentration in skin of an ornamental fish *Trichogaster lalius* is one of the candidate species for tropical aquariums.

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. 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 in to 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 (A-2), 4 g/kg (A-4), 6 g/kg (A-6) feed and one remained as control. The feed ingredients used to prepare the experimental diet provided in the 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

2.5 Observation of carotenoid content

For estimation of total carotenoid content in the skin of tested fish, procedure was followed as described by (Harpaz, and Padowicz, 2007) [11] with following equation: 4 × Optical density value × total volume of sample taken / weight of sample (mg). Here the overall variation in carotenoid level of skin was observed at different spectrophotometric wave lengths (WL) *i.e.* 450 nm, 475 nm and 500 nm wave lengths.

2.6 Statistical analysis

The persistence rate of carotenoids in skin 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).

3. Results and discussion

Carotenoid concentration observed in skin at different web length

3.1 Observed at 450 nm wave length

The total variation in carotenoid level of skin was observed at 450 nm as shown in (Figure 1). It was found that the initial carotenoid concentration in skin was 0.1 µg/g but at the end of the experiment the carotenoid level went up to 6 µg/g. Maximum carotenoid accumulation was observed in skin of those fish which fed on 2g/kg spirulina powder incorporated diet (2-A) followed by 4g/kg (4-A) and 6g/kg (6-A) spirulina powder incorporated diet. A gradual degradation in carotenoid concentration of skin was observed in control fish (C-A) which were not provided spirulina powder in their diet (Figure: 1).

3.2 Observed at 475 nm wave length

Total carotenoid concentration in skin of *T. lalius* was also observed in 475nm wavelength as shown in (Figure: 2). The obtained results indicated that all three concentration of spirulina powder incorporated diet increased total carotenoid concentration of skin. The initial carotenoid value in skin was 0.05µg/g and the final carotenoid value was 0.35µg/g. The maximum carotenoid accumulation was observed on 45^{th} day and 60^{th} day of sampling in those fishes which were provided with 6g/kg spirulina powder incorporated diet (Figure 2).

3.3 Observed at 500 nm wave length

The overall variation in carotenoid level of skin was observed at 500nm as shown in (Figure 3). It was ranged from 0.04 to 1.16µg/g. A maximum amount of carotenoid was recorded in 2g/kg followed by 6g/kg spirulina powder incorporating diet fed fish and minimum level of carotenoids was seen in 4g/kg spirulina and control diet fed fish (Figure: 3).The impact of different doses of spirulina powder in diet on skin colouration was statistically calculated by using statistical methods.

At the end of experiment, it was evaluated that supplementation of spirulina powder in the diet significantly increased (P<0.05) the total carotenoid concentration in the skin of dwarf gourami whereas absence of spirulina powder in diet (control) caused a significant reduction (P<0.05) in carotenoid level of skin of dwarf gourami.

The result showed that spirulina powder incorporated diet had an impact on skin colouration of *T. lalius*. Most effective dose of spirulina powder which was responsible for significant change in skin colouration was determined by student "t-Test". There was significant increased (P<0.05) in carotenoid concentration in all three treatments at different wave lengths compare to control.

The result obtained from t-Test showed that 2g/kg spirulina powder incorporated feed increased the skin carotenoid concentration significantly (p<0.05) highest at 450 nm and 500 nm wave length respectively compare to other two treatments and control. But at wave length of 475 nm, it was found that 6g/kg spirulina powder incorporated diet responded maximum carotenoid accumulation in skin compare to other two treatments and control.

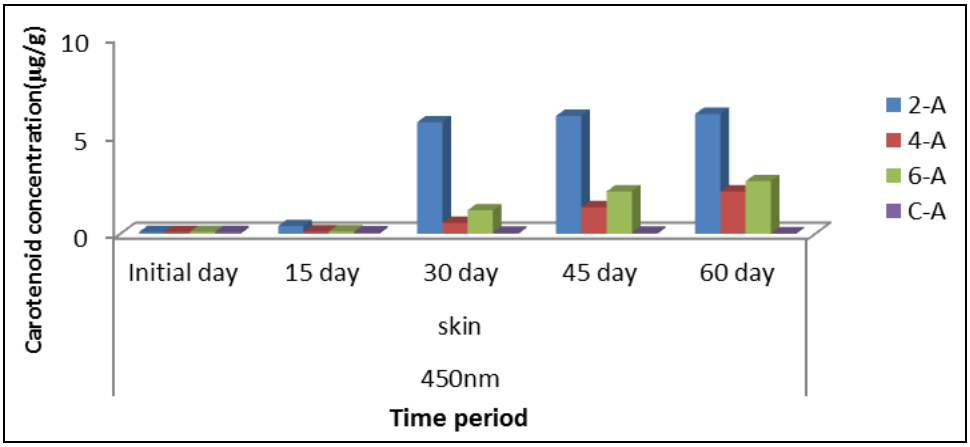

Fig 1: Carotenoid concentration in skin (at 450 nm WL) of *T. lalius* treated with different concentrations of spirulina incorporated feed and control feed

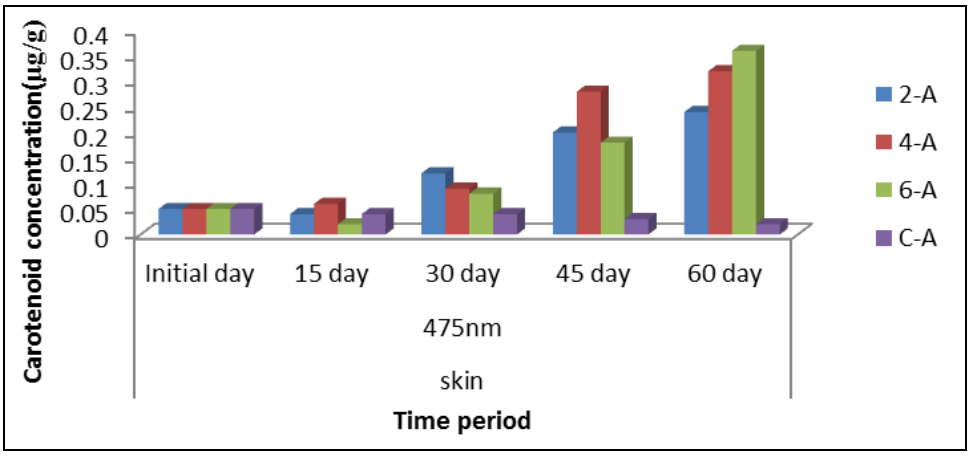

Fig 2: Carotenoid concentration in skin (at 475 nm WL) of *T. lalius* treated with different concentrations of spirulina incorporated feed and control feed

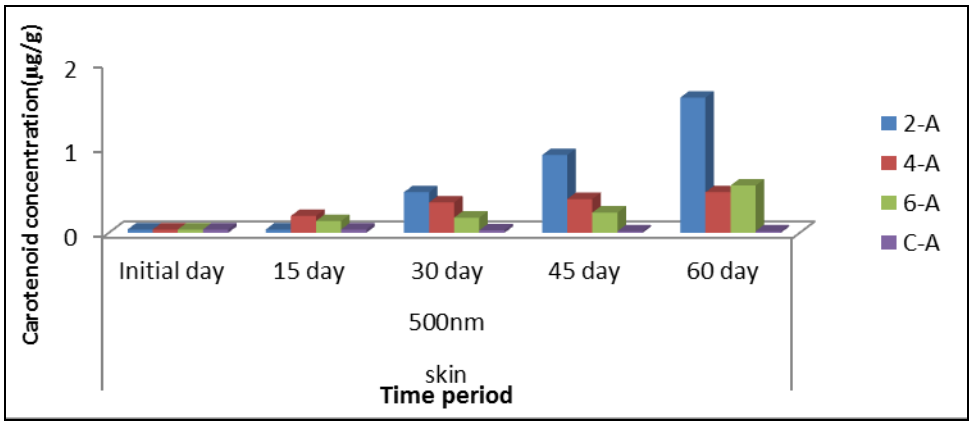

Fig 3: Carotenoid concentration in skin (at 500 nm WL) of *T. lalius* treated with different concentrations of spirulina incorporated feed and control feed

Natural carotenoids use to provide better colouration among fishes. The source of carotenoid content may be come from flora and fauna residue which can enhance the body colour in fishes. Ahilan *et al.* (2008) [1] reported the effect of 3 botanical additives (Coriander, Mint and Amaranth leaves) on the growth and body colouration of gold fish. Joseph *et al.* (2011) [14] reported that carotenoids of *Hibiscus rosa-sinensis*, induced pigmentation to make orange sword tail more colourfull. Sinha and Asimi (2007) [23] also reported the same that the China rose (*Hibiscus rosa-sinensis*) petal is a potent natural carotenoid source of gold fish (*Carassius auratus*) to enhance its colour. Vasudhevan *et al.* (2013) [26] also conducted experiment on gold fish and found that 50 g *Azolla* per kg provide more carotenoid deposition in muscle. Other plant based sources like red pepper (Yılmaz and Ergün, 2011) [29] and marigold flower (Yanar *et al.*, 2007) [27] have been tested as dietary sources for pigmentation of the skin with different results. Ezhil *et al.* (2013) [8] reported that encouraging results on colouration of red sword tails (*Xiphophorous helleri*) fed diet containing marigold petal meal. Alagappan *et al.* (2004) [3] also reported the influence of carotenoid value in skin of blue gourami**.**

Not only for the plant based sources, are animal sources also now very much effective to enhance the body colour of the ornamental fishes. Tanaka *et al.* (1996) [25] reported that by using crab waste as a carotenoid source enhance the colour development in goldfish. Kamata *et al.* (1990) [15] observed the colour development in rainbow trout when fed with *Adonis aestivalis* as a pigment source. Similarly, marine products such as shrimp meal (Diler and Gokoglu, 2004) [7] and krill meal have been evaluated as pigment sources in diets for ornamental fish (Yılmaz and Ergün, 2011) [29] and trout (Roncarati *et al.*, 2011; Hernández *et al.*, 2012) [22, 12].

Spirulina powder was used as colour enhancer by many scientists(Boonyaratpalin and Unprasert, 1989; Mori *et al.*, 1987; Okada *et al.*, 1991; Sommer *et al.*, 1992; Choubert, 1979; Yesilayer *et al.*, 2011; Ezhil and Narayanan, 2013) [5, 17, 18, 24, 6, 28, 8].Several author (Arulvasu *et al.*, 2013; Ramamoorthy *et al.*, 2010; Ahilan *et al.*, 2008; Harpaz and Padowicz, 2007) [4, 21, 1, 11] also used natural carotenoid source to increase colouration of swordtail, *Xiphophorus hellerii*, Amphiprion *ocellaris*, *Carassius auratus* and Dwarf Cichlid *Microgeophagus ramirezi.* The present investigation coincides with Ako *et al.*, 2000 [2] who observed enhanced pigmentation in topaz cichlids, *Cichlasoma myrnae* when fed a diet containing 1.5-2.0 g of a carotenoid-rich strain of *Spirulina platensis*.

The result of the present experiment suggested that dietary inclusion of 2g/kg spirulina powder incorporated diet was responsible for increased in skin carotenoid concentration significantly (P<0.05) at 450 nm and 500 nm wave length respectively. But at 6g/kg spirulina powder incorporated diet responded maximum carotenoid accumulation for the wave lengths of 475 nm in skin of *T. lalius*. 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.

4. Conclusion

Color is one of the major factors, which determines the price of aquarium fish in the world market. Attractive coloration determines the commercial value of ornamental fishes. Pigmentation in the skin is responsible for coloration in the fish. The colour enhancing diets contained additional natural pigments which enhances the colour of ornamental fish. Spirulina powder, easily available in local market and can be smoothly incorporate as feed ingredients. Spirulina powder not only useful in pigmentation in fishes but also enhanced growth efficiency in fishes and thus spirulina incorporated diets may be recommended in commercial culture to enhance the growth and pigmentation in dwarf gourami and other alike fishes.

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