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The Effects of Dietary Supplement of *Arthrospira platensis* (Spirulina) as a Fishmeal Replacer on Growth Performance and Carotenoid Concentration in Buenos Aires Tetra (*Aphyocharax anisitsi*)

Masud Rana[1], Mousumi Das[2], Sehnaz Parvin[1], Sk. Kabita[1]

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ABSTRACT

Background: *Arthrospira platensis* (Spirulina) is a potential ingredient in aquafeed for containing high protein, minerals, vitamins and pigments which provide nutritional value to fish. It is a growth promoter and carotenoid source in non-ornamental fishes.

Methods: To evaluate the potential of *A. platensis* (Spirulina) as a growth promoter and carotenoid source for transferring from the diets to the muscle and skin of Buenos Aires Tetra, *Aphyocharax anisitsi*, five experimental diets were prepared by replacing fishmeal, D0 (0%) (control), D25 (25%), D50 (50%), D75 (75%) and D100 (100%). The growth parameters like weight gain, length gain, specific growth rate and food conversion ratio were measured and carotenoid content in the muscle and skin of the fish was estimated.

Result: At the end of the experiment, significantly ($P<0.05$) higher weight gain (WG), length gain (LG), specific growth rate (SGR) and feed conversion ratio (FCR) were observed in D50 and D75 diet groups compared to control and D100 where fishmeal was replaced by 21 g and 31.5 g spirulina respectively. The carotenoid content in the muscle and skin was also found to be highest in D75 compared to control. The findings of this experiment showed that *A. platensis* could replace up to 50% and 75% fishmeal in the diet of *A. anisitsi* for optimum growth and carotenoid accumulation in the muscle and skin.

Key words: Buenos aires tetra, Carotenoid, Growth, Spirulina.

INTRODUCTION

Fishmeal is an essential ingredient as a protein source in the diet of fish (FAO, 2016). Although fishmeal is most nutritious and easily digestible ingredient, use of it in high amounts is not sustainable due to its high price. For this reason, an alternative feed ingredient which contains high protein is needed to replace fishmeal. *Arthrospira platensis* (spirulina) a microalga, is an alternative plant-derived protein source that could replace costly animal-derived protein. Research has shown that consumption of spirulina improves growth performance in several fish species like Coral trout, *Plectropomus leopardus* (Yu *et al*., 2018), great sturgeon, *Huso huso* (Adel *et al*., 2016), gibel carp , *Carassius auratus gibelio* var. CAS III (Cao *et al*., 2018), Queen loach, *Botia dario* (Gogoi *et al*., 2018), Nile tilapia, *Oreochromis niloticus* (Elabd *et al*., 2020; Shalata *et al*., 2021), blunt snout bream, *Megalobrama amblycephala* (Jiang *et al*., 2022), Catfish, *Clarias gariepinus* (Rosenau *et al*., 2021). The excellent nutritional value of spirulina makes it alternative protein source and growth promoter due to its high protein, diverse amino acid (AA) and fatty acid (FA), vitamins, minerals and several bioactive molecules. In addition, Spirulina has been proven to be an excellent feed attractant in shrimp in very low inclusion in diets (Silva-Neto *et al*., 2012). There are many research works available about effect of spirulina on growth performances in non-ornamental fish species and these have been used to determine the effect of spirulina on growth and colouration of ornamental fish. That's why there is a lack of information about the nutrient needs of ornamental fish.

The skin color of ornamental fish is the most important parameter determining market price and demand. The beauty and coloration of ornamental fishes are caused mainly by the presence of chromatophores in the skin, which possesses pigments and light-producing substances (Bisht *et al*., 2023). Carotenoids impart orange, red and yellow colour on fish (Colihueque, 2010; Kop and Durmaz, 2008). When ornamental fish are kept in aquariums or captivity, the colour

[1]Department of Biological Sciences, Aliah University, Kolkata-700 160, West Bengal, India.
[2]Department of Zoology, Vidyasagar College, University of Calcutta, Kolkata-700 073, West Bengal, India.

Corresponding Author: Sk. Kabita, Department of Biological Sciences, Aliah University, Kolkata-700 160, West Bengal, India. Email: kabita@aliah.ac.in

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fades and decrease consumer acceptability. Fishes cannot synthesize de novo or to convert absorbed food material to colouring agents (Chatzifotis *et al*., 2005). They rely on plants and algae to gain these products. Researchers have emphasized improving the coloration of ornamental fish by using natural source of colouring agents. *A. platensis*, a microalgae have very thin cell wall that can be easily digestible by fish digestive enzymes (Roohani *et al*., 2019).

Spirulina have been used as natural source of carotenoids to prepare diet for several fish species like Mekong Giant Catfish (Pla Buk), *Pangasianodon gigas* (Tongsiri *et al*., 2010), *Cyprinus carpio* (Ansarifard *et al*., 2018), Oscar fish, *Astronotus ocellatus* (Mohammadiazarm *et al*., 2021), yellow catfish, (Liu *et al*., 2019), Caspian brown trout, *Salmo trutta caspius* (Meshkat Roohani *et al*., 2020), that positively enhance the skin and fillet carotenoid and coloration. 52.8 g/kg spirulina has been found to enhance colouration in Caspian brown trout, *Salmo trutta caspius* (Meshkat Roohani *et al*., 2020). As the growth and carotenoid concentration have been seen in different non-ornamental fishes by feeding spirulina diet, the ornamental fish Buenos Aires Tetra *A. anisitsi* have been selected for this study. In addition, no research has been conducted on the impact of A. platensis on growth performance and the efficiency of carotenoid deposition in the tissue and skin of *A. anisitsi*. This fish is also a very popular ornamental fish, therefore doing research on the eco-friendly and suitable diets is essential.

MATERIALS AND METHODS

Diet preparation

Five diets (D0, D25, D50, D75, D100) which were isonitrogenous were formulated by replacing 0%, 25%, 50%, 75%, 100% of dietary fishmeal with spirulina powder (Table 1). All the five formulated diets are isonitrogenous. Diets are formulated according to Pearson square method. Crude protein of all the ingredients were determined by Kjeldahl method, using Kjeldahl apparatus my measuring nitrogen content. After preparing all the experimental diets, their protein contents were estimated by the same procedure. The ingredients of the diet were finely ground and prepared dough by mixing ingredients with required amount of water at room temperature. Then the dough pass through meat mincer with 1mm diameter. The pellets were air dried and stored in plastic bags at -20°C until used.

Experimental fish

Before feeding trial, all fish were kept in a glass aquarium (200L) and fed control feed for 14 days by hand twice a day for acclimatization (10:30 and 17:00) in laboratory condition. At the beginning of the experiment, healthy and similar were randomly selected and stocked into 5 glass aquaria (D0, D25, D50, D75, D100) at a density of 10 fish per aquarium in triplicate. During the trials, the experimental food was fed by hand daily twice (10:30 and 17:00) at the rate of 2% of their body weight (*ad lib*) and ration was changed according to mean fish weight change after each 30 days interval. The tenure of feeding trial was 120 days (June-September, 2022) and the experiment was carried out at the department of Biological sciences, Aliah University, Kolkata. The water was kept at a constant temperature of 30±5°C, dissolved oxygen (DO) content was maintained above 6 mg/L by flowing air and the ammonia was less than 0.1 mg/L and pH was maintained 7.4. The quality of water from each experimental aquarium was analysed twice a week using the standard method (APHA, 1989).

Growth parameters

The growth performance parameters like weight gain (WG,%), length gain (LG,%), specific growth rate (SGR, %/day) and feed conversion ratio (FCR) were calculated according to the following formulae (Liao *et al*., 2022; Mohammadiazarm *et al*., 2021; Roohani *et al*., 2019).

Weight gain (WG, %) =

$$\frac{\text{Final fish weight } (W_f) - \text{Initial fish weight } (W_i)}{\text{Initial fish weight}} \times 100$$

Length gain (LG, %) =

$$\frac{\text{Final fish length } (L_f) - \text{Initial fish length } (L_i)}{\text{Initial fish length}} \times 100$$

Specific growth rate (SGR, %/day): It is the percentage increase in weight per fish per day.

Table 1: Formulation and ingredients of experimental diets.

Ingredients	Diets				
	D0	D25	D50	D75	D100
Vitamins and minerals	2	2	2	2	2
Fishmeal (FM)	42	31.5	20	10.5	0
Arthrospira platensis	0	10.5	21	31.5	42
Soybean meal	20	21	20	20	20
Wheat flour	15	15	15	15	15
Mustard oil cake (MOC)	9.28	6.73	4.19	1.64	0.9
Corn starch	5.5	1.77	9.04	16.31	23.58

Dietary treatments with graded levels of fish meal replacement with *A. platensis*: control, 0% D0 (0 g), 25%, D25 (10.5 g), 50%, D50 (21 g), 75%, D75 (31.5), 100%, D100 (42 g).

$$SGR = \frac{100 \times (\ln \text{final body weight, } W_f - \ln \text{initial body weight, } W_i)}{\text{No. of days}}$$

Feed intake = The total of the consumed feed (g) by fish during the rearing period.

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed consumed (g)}}{\text{Final body weight (g) - Initial body weight (g)}}$$

Carotenoid content

The amount of carotenoid accumulated in the skin and muscle was analysed by following the method of (Olson, 1979). 1 gram tissue of *A. anisitsi* was taken in a 10 ml glass vials. Then 2.5 g anhydrous sodium sulphate was added into this vial and mixed gently. 5 ml chloroform was added and kept overnight at 0°C to form a clear layer of chloroform on the upper portion of vial. 0.3 ml aliquots diluted to 3 ml absolute ethanol and optical density was measured at 380, 450, 470 and 500 nm in spectrophotometer.

Statistical analysis

The collected data which were obtained throughout experiment tenure were analysed by one way analysis of variance (ANOVA) with a significant level of ($P<0.05$).

RESULTS AND DISCUSSION

Growth performance

Table 2 represents the weight gain (WG), length gain (LG), specific growth rate (SGR) and feed conversion ratio (FCR) of *A. anisitsi* during the experimental tenure. The highest and lowest weight gain and length gain was found in the diet group D75 and D100 respectively. The SGR was also found highest in the diet group D75 and the lowest SGR was resulted in the diet group D100.

The diet group D100 found to be poor performing diet group than the control group D0. *A. anisitsi* fed with 25%, 50%, 75% spirulina supplemented diet showed increasing trend in WG, LG, SGR than the control diet group. However, complete replacement of fishmeal by *A. platensis* manifested negative effect on these parameters and this diet group showed very poor performance even lower than the control. The best feed conversion ratio (FCR) was recorded in the diet group D75 containing 75% of spirulina followed by the diet group D50 containing 50% spirulina. The lowest FCR was recorded in the diet group D100 containing 100% spirulina that was even lower than the control diet.

These result of our study is in agreement with the study of (Velasquez *et al*., 2016) and (El-Sheekh *et al*., 2014) where 75% fishmeal replaced by *A. platensis* enhanced WG, LG, SGR, FCR in tilapia, *Oreochromis niloticus* and hybrid red tilapia, *O. niloticus* x *O. mossambicus* respectively. But the results of this experiment contradict with (Nandeesha *et al*., 1998, 2001), where *Cyprinus carpio* and *Catla catla* were fed 25%, 50%, 75% and 100% spirulina supplemented diets and WG, SGR, FCR was not affected significantly whereas 100% fishmeal replacement by spirulina in the diets of *Labeo rohita* showed significantly higher growth rate. In the present study, 25-50% inclusion of spirulina diet increases SGR, WG, LG and FCR. Hussein *et al*. (2013) showed that 50% spirulina diet (D50) increases growth (SGR, WG, FCR), but 100% spirulina (D100) depressed these parameters and these results are similar with our study. In the present study, no further increase in the growth performance of *A. anisitsi* was achieved by increasing spirulina inclusion over 75%.

Table 2: The growth performance (mean±SE) of the experimental fish after feeding with five different diets.

Growth parameters	Experimental diets				
	D0	D25	D50	D75	D100
Initial weight (g)	0.22±0.01^a	0.22±0.00^a	0.22±0.005^a	0.21±0.007^a	0.21±0.004^a
Final weight (g)	1.72±0.06^a	2.23±0.05c	2.93±0.09^d	3.35±0.07^e	1.25±0.07^a
Initial length (cm)	2.37±0.03^a	2.33± 0.03^a	2.3±0^a	2.4±0^a	2.4 ± 0^a
Final length (cm)	5.28±0.04^b	5.58±0.02^c	5.7±0^d	5.58±0.02^e	4.97±0.03^a
Length gain (LG, %)	2.92±0.02^b	3.25±0.03^c	3.4±0^d	3.48±0.02^e	2.57±0.03^a
Weight gain (WG, %)	1.51±0.05^b	2.01±0.04^c	2.71±0.09^d	3.14±0.07^e	1.04±0.07^a
Food consumption/fish (g)	2.64±0.03^b	2.95±0.03^c	3.38±0.03^e	3.21±0.03^d	2.19±0.04^a
Specific growth rate (%/day)	3.46±0.03^b	3.85± 0.03^c	4.36±0.013^d	4.61±0.04^e	2.96±0.07^a
Food conversion ratio (FCR)	1.75±0.04^d	1.47±0.01^c	1.25±0.03^b	1.03±0.01^c	2.12±0.1^e

Mean values with different superscripts in the same row are significantly different. The significance level is defined as $P<0.05$.

Table 3: Amount of carotenoid deposition in muscle and skin (µg/g) in *A. anisitsi* fed with five different diets.

Diets	Carotenoids in food (µg/g)	Carotenoid in muscle and skin (µg/g)
D0	0.04±0.002	1.0266±0.074
D25	0.074667±0.001	2.386667±0.116
D50	0.108±0.002	5.36±0.092
D75	0.146667±0.003	9.2933±0.058
D100	0.185333±0.004	1.49333±0.081

This may be due to reduction of palatability and lack of phosphorus availability (Nandeesha *et al*., 2001; Nandeesha *et al*., 1998; Olvera-Novoa *et al*., 1998; Palmegiano *et al*., 2008). Moreover, *A. anisitsi* fed with 25%-75% spirulina inclusion level showed better growth performance as compared to control as well as D100. This means, spirulina inclusion in the diet of *A. anisitsi* will be most suitable for its optimum growth around 50%-75% and according to our study the 75% spirulina diet was best assimilated by *A. anisitsi* than control.

Carotenoid content

Table 3 represents the carotenoid content in the five different diets and the tissue and skin. The results of this study showed that carotenoid deposition in the muscle and skin of *A. anisitsi* was increased with increasing *A. platensis* inclusion in the diet up to 75%. Although D100 diet contain highest carotenoid (0.1853±0.0048 µg/g), the carotenoid deposition in this group is poorly occurred. In the present study, carotenoid content in the skin and muscle showed increasing trend when spirulina increases from 25%-75% in the diet (D25-D75) compared to control, no further increase in carotenoid accumulation was observed beyond the diet group D75 but 100% spirulina inclusion had drastically diminished the accumulation of carotenoid in the muscle and skin (Table 3). The highest carotenoid accumulation was observed in D75 diet group because *A. anisitsi* consumed highest amount of this diet and utilized maximally (Table 2). The results of this study showed concentration of carotenoid in the diet and skin and muscle linearly correlated for the diet group D0-D75 group, but D100 did not follow this linear relationship. According to the present study, 50% and 75% spirulina inclusion to the feed of *A. anisitsi* were efficient for maximum carotenoid deposition as these diets contain higher carotenoid content compared to control. In these two diet groups, the carotenoid deposition was greater because *A. anisitsi* utilized these two diets efficiently. Colouration in fish is influenced by the carotenoid content in *A. platensis* which contain many types carotenoids like xanthophyll (yellow), zeaxanthin, β-carotene (orange)(Gouveia *et al*., 2003) and it is digested, transported and stored in the skin and muscle. The changes in the colouration in our study were observed by measuring the elevated total carotenoid content in the muscle and skin of *A. anisitsi*.

CONCLUSION

The results of this study showed that spirulina could be used in the diet of *A. anisitsi* to replace up to 50% and 75% fishmeal without causing any adverse effect on growth and colouration. These two spirulina supplemented diets (D50 and D75) causes higher growth values and carotenoid deposition in this fish. *A. platensis* meal has the potential for using as alternative to the fishmeal for *A. anisitsi* and this preliminary study on this fish species confirms a basis for further extensive investigation.

Conflict of interest

All authors declare that they have no conflicts of interest.

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