Aquaculture Studies, 23(2), AQUAST931
http://doi.org/10.4194/AQUAST931

RESEARCH PAPER

Effect of Spirulina Powder (*Arthrospira platensis*) as a Dietary Additives Ornamental Guppy, *Poecilia Reticulata:* Growth Performance, Survival and Skin Colouration

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How to Cite

Bisht, M., Kumar, A., Kumar Shah, T., (2023). Effect of Spirulina Powder (*Arthrospira platensis*) As a Dietary Additives Ornamental Guppy, *Poecilia Reticulata:* Growth Performance, Survival and Skin Colouration. *Aquaculture Studies*, *23(2), AQUAST931*. http://doi.org/10.4194/AQUAST931

Article History
Received 05 April 2022
Accepted 26 August 2022
First Online 29 September 2022

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Keywords
Natural carotenoid
Guppy
Pigmentation
Skin coloration

Abstract

The present study was carried out to evaluate the effects of replacing fish meal with dietary *Arthrospira platensis* as a feed supplementation on growth performance, survival and skin pigmentation of guppy, *Poecilia reticulata*. A total number of two hundred forty healthy fishes (average body weight 0.15±0.02) were equally divided into four feeding groups (triplicates) as such as control (T_0), 5% (ST_1), 10% (ST_2) and 15% (ST_3). The final experiment was conducted in aquarium for 60 days. The average crude protein and Crude lipid levels of the diet is 10.31 & 39.81%, respectively. A significant increase in the concentration of carotenoids in fish skin was observed with an increase in spirulina concentration in fish feed. However, the ST3 treatment had highest carotenoids concentration. Redness, yellowness and whiteness found to have significant differences. There was a significant difference between treatment groups concerning the growth parameters. The ST_3 diet significantly increases length, weight, net weight gain, weight gain, specific growth rate and survival rate. Inclusion of 15% Spirulina was found to be suitable to ensure growth as well as pigmentation of guppy. Spirulina powder can be used as protein and carotenoid supplements in fish feed to improve growth, survival and colouration of guppy.

Introduction

Ornamental fishes are beautiful pets compared to many other pet animals that are maintained in aquaria (Biswas et al. 2007). Keeping colourful tropical fishes in aquarium and garden ponds is a hobby since centuries by humans. The global ornamental fishes export is also escalating in marine and freshwater fish valued at US$ 337 million in the year 2017 (Anonymus, 2019). Colour is one of the major factors, which determine the value of ornamental fish in the global market (Saxena, 1994). The body colour of fish predominantly dependent on the presence of chromatophores on the skin which contain pigments and light-reflecting organelles. Carotenoids are lipid soluble yellow to red in coloured pigments and in the muscle (flesh), the carotenoids are the most important pigments (Dernekbasi et al. 2010). However, carotenoids cannot be biosynthesized by animals and fishes, therefore, they must be obtained from the dietary source (Booth et al. 2004).

However, detailed studies of colour enhancement of ornamental fish by using pigment enriched fish feed are lacking. Many algal species are used as the nutritional source in aquaculture (Muller-Feuga, 2000). Spirulina is one of the most important aquatic animal feed ingredient and most commonly used in aquaculture industry because its high contents of protein, vitamins, essential amino acids, minerals, essential fatty acids and antioxidant pigments such as carotenoids (Nakagawa and Montgomery, 2007). Spirulina, *Spirulina platensis* (SP), is a strain of cyanobacteria (blue-green algae) that can be used because of nutritional benefits, for example protein, amino acid, vitamins, minerals, essential fatty acid and β-carotene in aquafeeds (Zhang et al., 2019).

High protein content percentage of spirulina and its well-balanced amino acid profile compared with other plant protein sources makes it as potential fish meal replacer in the aquafeed formulation. Different types of synthetic carotenoids, such as astaxanthin, canthaxanthin and lutein (Hanel et al. 2007), as well as natural carotenoid sources have been used as dietary supplements to increase the colouration and pigmentation of ornamental fish (Gouveia and Rema, 2005). Guppies (*Poecilia reticulata*) are widely preferred fish and are also one of the commonly reared aquarium fish worldwide. They belong to the family Poeciliidae and are widely distributed tropical fish which are also known as million fish and rainbow fish (Shah et al. 2017).

The present study the effects of carotenoids extracted from spirulina on the growth parameters of fish and their influence on skin colouration was studied. The main objective of the present study was to compare different dose of S*. platensis* on growth parameters and skin colouration of guppy.

Materials and Methods

Fish, Experimental Design and Condition

Guppies were purchased from a local commercial aquarium fish supplier, who procured them from Kolkata and kept for acclimatization under laboratory conditions for 15 days. The final experiment was conducted in aquarium (4.0 x 1.0 x 1.0 feet) for 90 days. Two hundred forty healthy fishes (average body weight of 0.15±0.02 g) were collected from the acclimatized tank and stocked in aquarium for the experiment. Guppies were divided into four treatments (three replicates per treatment), having 60 fishes in each treatment (20 fish in each replicates).

Experimental Diet

Four different diets were formulated containing different levels of spirulina for comparative study. The spirulina was incorporated into the diet as: control (T_0), 5% (ST_1), 10% (ST_2) and 15% (ST_3). A diet with and without spirulina powder are used as a treatment group & control group, respectively.

Sampling

All the fishes in each treatment group were weighed (per individual) and skin colour was measured at the beginning and end of the experiment.

Proximate Composition of Diet

The composition of different ingredients used in the diet is shown in Table 1. All ingredients were collected from fish feed unit (College of Fisheries, Pantnagar). Proximate composition of fish feed was done by the methods of Association of Official Agricultural Chemists [AOAC] (2005). The feed ingredients were then mixed in the desired proportion using trial and error method with Peterson square. They were then steam cooked separately, dried and pelleted.

Growth performance

Periodic sampling was done fortnightly for monitoring the growth performance.

$$\text{Weight gain (\%)} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} \times 100$$

$$\text{Net weight gain (g)} = \text{Final Weight} - \text{Initial Weight}$$

$$\text{SGR (\%)} = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{Duration of Experiment (days)}} \times 100$$

SGR: Specific Growth Rate

$$\text{FCR(g/g)} = \frac{\text{feed given (Dry weight)}}{\text{Body weight gain (Wet weight)}}$$

$$\text{Survival rate (\%)} = \frac{\text{Total fish harvested}}{\text{Total fish stocked}} \times 100$$

Table 1: Percent and Proximate composition of control and experimental diets

Ingredients	Dietary group (%)			
	Control Diet	Spirulina Diet		
	T_0	ST_1	ST_2	ST_3
Fish meal	35.20	31.05	26.90	22.85
Rice bran	41.50	40.65	39.80	38.85
Soya bean meal	22.30	22.30	22.30	22.30
Premix Vitamins + Minerals	1.00	1.00	1.00	1.00
Spirulina Powder	0.00	5.00	10.00	15.00
Proximate Composition				
Moisture (%)	8.56±0.31	8.46±0.12	8.37±0.14	8.19±0.26
Crude Protein (%)	39.80±0.58	39.8±0.85	39.79±0.16	39.85±0.22
Lipid (%)	10.50±0.18	10.41±0.15	10.32±0.26	10.04±0.16
Ash (%)	10.07±0.23	9.78±0.20	9.48±0.20	9.11±0.23
Crude fibre (%)	9.63±0.16	9.64±0.30	9.66±0.21	9.45±0.27

Colour Evaluation

Two methods were used to evaluate the colour of fishes: (a) total carotenoid analysis; and (b) determination of skin colour by Spectrophotometer

Total Carotenoid Analysis

Analysis of total carotenoids in the fish tissue and feed sample was done by the methods described by Olson, 1979 with some modification. Fish body tissue (without head and alimentary canal) or feed sample was weighted and 1 gram was taken in a screw capped clean glass vial of 10 ml and 2.5 g of anhydrous sodium sulphate was added to that vial. The sample was then mashed gently inside the vial with a glass rod and 5 ml chloroform was added to that vial and was left overnight at 0°C in a freezer. A 1-2 cm of the clear layer was formed by the chloroform, and then 0.3 ml of aliquots of chloroform was separated and diluted with absolute ethanol to 3 ml. In a UV-VIS spectrophotometer (Systronic, digital 108) optical density was read at 380, 450, 470 and 500 nm. Similarly, a blank vial was prepared without any sample for the comparison of the optical density. The maximum absorption wavelength was used for the calculation of optical density.

$$\text{Total carotenoids content} = \frac{\text{Total fish Absorption at maximum wavelength harvested}}{0.25 \times \text{sample weight (g)}} \times 100$$

Determination of Skin Colour by Spectrophotometer

The skin colour of fish was measured by the method described by Gogoi et al., 2017. The skin colour was measure by using a Hunter Lab Spectrophotometer and calibrated to the white and black standard site of the fish sample. L*a*b* tristimulus measurement was used as it related to the human eye's response to colour. Representation of L* variable is skin lightness colour (L*=0 for black, L* = 100 for white), a* scale presented the red/green (+a* intensity in red and -a* intensity in green), and the b* scale represents the yellow/ blue (+b* intensity in yellow and -b* intensity in blue). The following equation to calculate of the whiteness, redness and yellowness (NFI, 1991):

$$\text{Whiteness} = 100 - [(100 - L*)^2 + a*^2 + b*^2]^{1/2}$$

$$\text{Redness} = \text{Cos}(16^\circ)\, a* + \text{Sin}(16^\circ)\, b*$$

$$\text{Yellowness} = -0.7\,[-\text{Sin}(16^\circ)\, a* + \text{Cos}(16^\circ)\, b*]$$

Statistical Analysis

The experiment was carried out under a completely randomized design. The analysis of variance (ANOVA) and test of significance was done by SPSS 16.0 (Gomez, 1984). All results were presented in Mean and standard error. The homogeneity of variances and normality of the residuals was tested using Levene tests and Shapiro-Wilks test, respectively. The level of significance used was $p<0.05$ from the table given by Fisher. Post hoc Tukey test was performed for comparison of groups.

Results

During the experiment periods, the effect of *Arthrospira platensis* powder on skin coloration of guppy was investigated. The concentration of carotenoid in fishes from different treatments with natural carotenoids supplemented with spirulina. The carotenoids concentration was also found to be significantly ($p<0.05$) highest in ST_3 followed by ST_2, ST_1 and lowest in T_0. The concentration of carotenoids increased with increased the spirulina concentration (Figure 1) and was also found to be significantly ($p<0.05$) highest in ST_3 followed by ST_2, ST_1 and lowest in T_0. However, significant difference were found among the carotenoid concentration (Figure 1). The skin colour was significant difference ($p<0.05$) for different parameters using different diets is shown in Table 2. The redness value was found to be significantly highest in ST3 (91.05 ± 0.10^{c}) followed by ST2 (76.48 ± 0.05^{ab}), and ST1 (69.68 ± 0.01^{b}) compared with control group, while

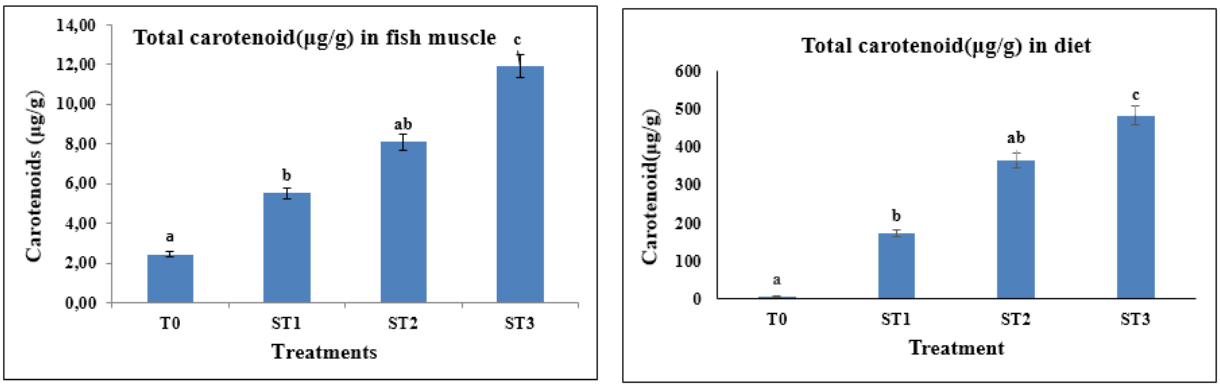

Figure 1: Effect of spirulina leaf powder on carotenoids in the fish tissue and feed sample of guppy. Values are means ± SD of three replications. Means in the same raw with different superscripts are significantly different ($p<0.05$)

yellowness value was found maximum in ST3 (17.38 ± 0.2^{c}) and minimum was found in ST_0 (15.23 ± 0.01^{a}). The whiteness value was found significantly highest in ST_3 (12.29 ± 0.05^{c}), followed by ST_2 (10.95 ± 0.02^{ab}), and ST_1 (8.90 ± 0.10^{b}) compared with the control group.

However, there was significant difference ($p<0.05$) in growth parameters such as final weight, weight gain, net weight gain, feed conversion ratio and specific growth rate (Table 3). Significantly ($p<0.05$) highest final mean weight was found in ST_3 (0.78 ± 0.01^{c}) while lowest in T_0 (0.60 ± 0.01^{a}). Final weight gain was found to be significantly ($p<0.05$) highest in ST_3 (499.26 ± 0.05^{c}) followed by ST_2 (446.15 ± 0.01^{ab}), ST_1 (391.23 ± 0.02^{b}) and lowest in T_0 (361.53 ± 0.01^{a}). Significantly ($p<0.05$) highest net weight gain was found in ST3 (0.65 ± 0.01^{c}) while lowest in ST_0 (0.47 ± 0.01^{a}). The specific growth rate was also found to be significantly ($p<0.05$) highest in ST_3 (0.86 ± 0.01^{c}) followed by ST_2 (0.82 ± 0.01^{c}) and lowest in T_0 (0.74 ± 0.01^{a}). Significantly ($p<0.05$) highest feed conversion ratio was found in T_0 (3.28 ± 0.10^{c}) while lowest in ST_3 (2.91 ± 0.04^{a}). No significant ($p>0.05$) difference was observed in survival rate, survival rate was found to be highest in ST_3 (78.33 ± 4.41^{c}) followed by ST_2 (76.67 ± 6.01^{ab}), ST_1 (73.33 ± 4.41^{b}) and lowest in T_0 (65.33 ± 1.35^{a}).

Discussion

To give desirable colour to the organism and its flesh incorporation of the carotenoids have been done as pigments in the diet of salmonids, crustaceans ornamental and other farmed fishes. Consumers generally relate the feed colour to the nutritive value, freshness, and taste of the feed. Hence, it has become an important factor in marketing. In the present study, results showed that supplementation of diet with *spirulina* powder had significant effects on carotenoids content. Similar findings were reported by Serdar et al. (2014), in which they fed red zebra cichlids (*Maylandia estherae*) with different carotenoid sources and found Spirulina and lutin diets increased the orange and yellow tint and astaxanthin diets increased the red-orange colour in the skin of the red zebra cichlid. For the development of pigmentation, such as the bright and attracting colours of ornamental fish carotenoids are used in supplementary feeds. Similar results were obtained by Yeşilayer et al. (2019) where the plant extract supplementations as natural carotenoid source yielded sufficient pigmentation on skin of electric yellow cichlids. Likewise, James et al. (2006) reported that the maximum carotenoid content was obtained in ornamental red swordtail (*Xiphophorus helleri*) fed with 8% spirulina. Skin coloration is one of the most important factors which decide the aesthetic value, therefore the market value of ornamental fishes. In the present study, there was significant difference observed in colour parameter. The redness and yellowness value generally shows the best relationship with the carotenoids as it increases as the carotenoid content increases (Bjerkeng, 2000), but in the present study, comparative value of redness and yellowness was found highest (ST3) and lowest (ST0).

The present experiment indicates that supplementation of spirulina powder in experimental guppy optimized growth parameters (weight gain, net weight gain, and specific growth rate) in 90 days experiment. Guppy fed with diets containing different concentrations of spirulina powder did show significant changes in their growth performance in present experiment. Several studies have been conducted about growth performance and skin colouration of culture fishes. Final weight, weight gain, net weight gain, feed conversion ratio and specific growth rate were non-significant using spirulina powder added to feed ($p<0.05$). Similar results were obtained by Bisht et al., 2021 where the *M. oleifera* leaf powder used as a supplementations. However, highest growth rate was observed for ST3 treatment where diet was formulated by incorporating 15% of spirulina. Good quality and

Table 2: Different colour measurement parameters in guppy at the end of the experiment

Parameter	T0	ST1	ST2	ST3
Redness	59.05 ± 0.02^{a}	69.68 ± 0.01^{b}	76.48 ± 0.05^{ab}	91.05 ± 0.10^{c}
Yellowness	15.23 ± 0.01^{a}	16.25 ± 0.50^{b}	16.98 ± 0.10^{b}	17.38 ± 0.2^{c}
Whiteness	6.34 ± 0.2^{a}	8.90 ± 0.10^{b}	10.95 ± 0.02^{ab}	12.29 ± 0.05^{c}

Values are means ± SD of three replications. Means in the same raw with different superscripts are significantly different ($P<0.05$).

Table 3: Effect of spirulina leaf powder on growth performance and survival of guppy

Diet	T_0	ST_1	ST_2	ST_3
Initial weight (g)	0.13 ± 0.01^{a}	0.13 ± 0.01^{a}	0.13 ± 0.01^{a}	0.13 ± 0.01^{a}
Final weight (g)	0.60 ± 0.01^{a}	0.64 ± 0.01^{b}	0.71 ± 0.02^{ab}	0.78 ± 0.01^{c}
Weight gain (%)	361.53 ± 0.01^{a}	391.23 ± 0.02^{b}	446.15 ± 0.01^{ab}	499.26 ± 0.05^{c}
Net weight gain (g)	0.47 ± 0.01^{a}	0.51 ± 0.01^{b}	0.58 ± 0.02^{b}	0.65 ± 0.01^{c}
Survival (%)	65.33 ± 1.35^{a}	73.33 ± 4.41^{b}	76.67 ± 6.01^{ab}	78.33 ± 4.41^{c}
SGR (%)	0.74 ± 0.01^{a}	0.77 ± 0.01^{b}	0.82 ± 0.01^{c}	0.86 ± 0.01^{c}
FCR	3.28 ± 0.10^{c}	3.14 ± 0.05^{ab}	2.99 ± 0.10^{b}	2.91 ± 0.04^{a}

Values are means ± SD of three replications. Means in the same raw with different superscripts are significantly different ($P<0.05$).

quantity of food, enough available oxygen and low-stress factors are essential for good growth of the fish (Huet, 1975). The present finding on spirulina showed that they can replace the fish meal as protein source (Kim et al. 2013). Moreover, the growth rate increased with increased the level of spirulina in guppy (Bisht et al., 2020: Dernekbasi, 2010). Similarly, working on ornamental guppies fed spirulina, researchers did not find significant changes in the growth performance because the spirulina diet might have increased the anti-nutritional factors in experimental diet (Mohammadiazarm et al. 2020). No significant difference on catla at any level of spirulina form control in respect of final weight, specific growth rate and protein efficiency ratio (Nandeesha et al. 2001). In present study, significant ($p>0.05$) difference was observed in case of survival rate, survival rate was found to be highest in ST_3 group followed by ST_2, ST_1 and lowest in T_0 (control) group. The support of one of finding of Wang et al. (2006) where the significant difference in survival rate of ornamental fish (*Hyphessobrycon callistus*) between the treatments when carotenoids were incorporated.

There was no significant difference ($p>0.05$) of proximate composition as such as moisture, crude protein, lipid, ash, crude fibre and carotenoids between the treatment groups. As expected, the carotenoids content of the spirulina diet was different, indicating the carotenoids increase with increase the concentration of spirulina in diet. Abdulrahman and Ameen (2014) used spirulina powder as supplementary feed for guppy and found non-significant changes in the proximate composition compared to control.

Conclusion

In the present study, it was observed that spirulina are a good source of protein and carotenoids for guppy. Improvement in fish performance in term of skin pigmentation and growth was observed with the increase in spirulina concentration in the diet. From the present study, it can be concluded that spirulina at the rate of 15% can be incorporated into the guppy diet for getting the best results in terms of colour development, growth performance and survival rate of guppy.

Ethical Statement

Not applicable

Funding Information

Not funded

Author Contribution

Mayank Bisht: Investigation, Data Curation, Formal analysis. Avdhesh Kumar: Experiment administration, Formal analysis, supervision. Tarang Kumar Shah: Writing – Original Draft Preparation, Resources, Data Curation, Formal analysis, Writing – Review & Editing

Conflict of Interest

Not applicable

Acknowledgements

The authors are thankful to the Dean, College of Fisheries & Head, Department of Aquaculture, GBPUAT, Pantnagar for providing the necessary facilities for the study.

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