International Journal of Agriculture, Forestry and Fisheries
2018; 6(3): 56-64
http://www.openscienceonline.com/journal/ijaff
ISSN: 2381-4365 (Print); ISSN: 2381-4373 (Online)

Open Science

Dietary Supplementation of Raw *Spirulina platensis* Increases the Growth Performance and Body Colouration in Guppy Fish (*Poecilia reticulata*)

Piyushani Iresha Sarathchandra*, Anjula Sachintha Mahaliyana, Sepalika Chandrani Jayamanne

Department of Animal Science, Faculty of Animal Science and export Agriculture, Uva Wellassa University, Badulla, Sri Lanka

Email address

piyushani09@gmail.com (P. I. Sarathchandra)

*Corresponding author

To cite this article

Piyushani Iresha Sarathchandra, Anjula Sachintha Mahaliyana, Sepalika Chandrani Jayamanne. Dietary Supplementation of Raw *Spirulina platensis* Increases the Growth Performance and Body Colouration in Guppy Fish (*Poecilia reticulata*). *International Journal of Agriculture, Forestry and Fisheries*. Vol. 6, No. 3, 2018, pp. 56-64.

Received: September 16, 2018; **Accepted**: October 8, 2018; **Published**: December 20, 2018

Abstract

The present study investigated the effect of dietary supplementation of different percentages of raw *Spirulina platensis* (5, 10, 15, 20, and 25% w.w) on growth performance and colour enhancement in guppy Guppy fish red blonde (*Poecilia reticulata*). Dietary supplementation was conducted with a commonly used basic fish feed in the ornamental aquaculture sector and the same commercial feed (Prima 0®) was used as the control treatment. There were significantly high final mean body weight and mean body length ($p<0.05$) in the treatments that fish were fed with 5% raw *Spiruina platensis* incorporated diet. Fish fed with all the supplemented diets showed a significantly high mean length gain ($p<0.05$) compared to the control diet. There were no any significant differences among the specific growth rates ($p>0.05$) for all the treatments. All the supplemented diets showed a better feed conversion ratio compared to the control diet, yet there were no any significant differences ($p>0.05$) between the supplemented diets. There was a significant difference between the commercial feed and all the *Spiruina platensis* incorporated diets in terms of the colour intensity of the fish body ($p<0.05$). As per the results, incorporation of raw *Spiruina platensis* leads to improved growth performances and colour enhancement in guppy fish red blonde. However, the studied incorporation levels did not show significant differences for the studied parameters in the fish.

Keywords

Colour Enhancement, Dietary Supplementation, Growth Performance, *Poecilia Reticulata*, *Spirulina Platensis*

1. Introduction

Spirulina platensis is a commercially important micro algae due to its overall nutritional qualities and it has been used over centuries as human food, animal feed and as a dietary supplement. This micro algae can be used as a partial supplement or a complete replacement of the protein source in fish feed due to its high protein content [1]. In addition to the well-balanced major nutrients, *Spirulina platensis* contains essential amino acids, essential fatty acids, vitamins, minerals and pigments, which make this micro algae more suitable as a feed supplement for ornamental fish. Phycocynin (blue) and chlorophyll (green) pigments responsible for blue green colour of *Spirulina platensis* and these two pigments with other pigments such as xanthophylls, β-carotene, echinenone, phycocyanin, zeaxanthin and cryptoxanthin make this micro alga ideal to use as a colour enhancement agent for ornamental fish species [2-5].

Aquarium trade is a rapidly growing sector in the international pet market and ornamental aquaculture plays a vital role in supplying the production to cater the rising demand [6, 7]. Guppy fish *Poecilia reticulata* is an ornamental fish with high demand due to its easiness to rare and breed. Their diversity in the ornamental features such as fin shape and body colour have made them to one of the most popular ornamental fish among hobbyists. In addition, guppy fish are used as a biological model in a variety of experiments including feeding trials [8, 9, 10].

In the present context, world tend to use satisfactory alternative feed supplements in enhancing growth performances and colourations of ornamental fish. However, development of aquaculture is retained by financial limitations which caused by the increasing prices of fish meal and other protein sources of animal origin. Therefore, the identification and use of alternative proteins, mainly of plant origin, are considered a priority to the growth of aquaculture industry [11-13]. Many different algae species already play vital role in aquaculture. It is widely known that the addition of microalgae to larval fish culture delivers a number of benefits and *Spirulina platensis* is known for improving growth performance and enhancing skin pigmentation in fish [5]. It has been examined that a small amount of algae added to fish diet obviously improves fish growth, lipid metabolism, body composition, colour and disease resistance [14-16]. Hence, the aim of the present study was to determine the effects of different rates of raw *Spirulina platensis* (5%, 10%, 15%, 20%, 25% on wet weight basis) supplementation to a common commercial diet on the growth performance and colour enhancement in guppy fish (*Poecilia reticulata*); red blonde variety.

2. Materials and Methods

2.1. Maintenance of *Spirulina Platensis* Culture

Zarrouck's medium was prepared and used as the culture medium for *Spirulina platensis* as described by [17]. All the *Spirulina platensis* cultures were maintained at Algal Culture Unit, Aquaculture Laboratory, Uva Wellassa University of Sri Lanka. Pure strain of *Spirulina platensis* cultures were maintained in culture tubes and used to conduct mass culturing through intermediate cultures. Mass culturing was done in 4 L transparent plastic bottles. Culture bottles were covered from the top side by transparent polythene to avoid contaminations. Samples from each mass culture unit were observed under the microscope on weekly basis to identify contamination. Clumps formation and the colour of mass cultures were observed on daily basis. Throughout the study, photo period was maintained as 12 hours light: 12 hours dark. Temperature inside the algal culture unit was monitored daily. Manual agitation of mass culture bottles and pure strain culture tubes were done three times per day. Clump formation occurred after 14 days of starting the mass cultures and these clumps were harvested and used to prepare the treatment diets.

2.2. Experimental Design

Experimental study was conducted in indoor conditions and cleaned glass tanks (30 cm X 15 cm X 15 cm) were used as the experimental units to keep fish. The experiment consisted with five treatments (raw *Spirulina platensis* incorporated diets) and a control (Commercial feed Prima 0®). Each treatment was conducted in triplicates and the treatments were allocated by Complete Randomized Design (CRD). Red blonde male guppy fish were obtained from Ornamental Fish Breeding and Training Center, National Aquaculture Development Authority (NAQDA) - Rambadagalla, and were acclimatized for 07 days under laboratory conditions prior to use for the experiment. Fish (21-day old) were stocked as 10 individuals per tank and continuous aeration was provided to each tank. Water quality conditions in the tanks were maintained daily by siphoning 1/3 of water from the tank bottom followed by adding new water to compensate the removed amount. A complete water exchange in the tanks was done once per week. Water quality parameters such as pH, temperature and dissolved oxygen (DO; mg/L) were measured on daily basis. This study was carried out over 60 days to cover the growth I (21-day old to 50-day old) and Growth II (50-day old to 80-day old) stages of guppy fish.

2.3. Preparation of Feed and Feeding Strategy

Harvested raw *Spiruina platensis* clumps were rinsed with distilled water twice and homogenized manually before incorporating into the commercial feed. Popular and commonly used basic commercial feed in ornamental aquaculture sector in Sri Lanka was used as the control diet and the same diet was supplemented with raw *Spiruina platensis* in different percentages (5, 10, 15, 20, and 25% w.w) to prepare the treatment diets. Homogenized raw *Spiruina platensis* and the commercial feed were mixed together to prepare the treatment diets and carboxy methyl cellulose (CMC) was used as the binding agent. From each diet, 20 g of moist feed were prepared to use over one-week period and they were stored in a refrigerator at 4°C. Fish were fed twice a day at a rate of 10% from their body weight over 60 days.

2.4. Proximate Analysis

Protein content of all the diets were determined according to AOAC official methods of analysis 928.08 (AOAC, 2000) by Kjeldahl method. Moisture content of each diet was analyzed by (AOAC method 950.46; AOAC, 2000) oven drying method (at 105°C until reached to a constant weight). The oven dried samples were further utilized to determine the ash content by the dry ashing method (AOAC method 920.153; AOAC, 2000). The samples were subjected to 550°C in a furnace an overnight. All the diets were analyzed for their fat content by Soxhlet extraction followed by the gravimetric method (AOAC method 960.39; AOAC, 2000).

2.5. Carotenoid Content Analysis of Prepared Diets

Carotenoid pigment contents of all the diets were measured by UV spectrophotometry method. A sample of 2 g from each diet was transferred into 10 ml of absolute ethanol. After 6 hours, the solution was filtered by a filter paper and the extract was collected. Absorption of carotenoids was measured at the wave length of 453 nm. Carotenoid pigment

concentrations were calculated based on the Beer - Lambert law (A = ε λ × c × d).

2.6. Measuring Growth Parameters

Growth parameters including live body weight and length of each fish were measured at every two weeks interval over the study period. Weight of each fish in each tank was measured by an analytical balance (with a precision of 0.001 g) and length was measured by a digital vernier caliper (with a precision of 0.1 mm). Weight gain, length gain, specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (%) were measured as described elsewhere as follows [18].

Weight gain (g) = Mean final weight - Mean initial weight

Length gain (cm) = Mean final length (cm) - Mean initial length (cm)

$$\text{Feed conversion ratio (FCR)} = \frac{-b \pm \text{Feed (g) consumed by the fish}\sqrt{b^2 - 4ac}}{\text{Weight (g) gain of the fish (W2} - \text{W1)}}$$

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

2.7. Analysis of Colour Enhancement

Photographs of all 10 fish in each tank were taken before starting the feeding trials, after one month and after two months by Canon EOS 1300D camera to measure the intensity of their body colour. All photographs were taken with 90^0 angle with 1/100 second exposure time. Camera was used in 10 cm of distance above the specimens allowing the capturing of whole fish to the image. The images were analyzed by imageJ software [19]. Pictures were opened in RGB mode. Measurements were processed along the fish tail and close to the tail. In each position, five repetitions were recorded, and the average value was used to evaluate the colour enhancement [20].

2.8. Statistical Analysis

Significant differences among the treatments were identified by Tukey Test and one-way ANOVA at the significance level of p = 0.05. All the structured designs and data were analyzed using MS Excel 2007 and Minitab 17 software packages.

3. Results

3.1. Results of Proximate Analysis in Each Diet

As indicated in the Table 1, protein content and the fat content of the diets increased with the increase of the percentage of raw *Spirulina platensis* in the diet.

Table 1. *Protein, fat, moisture and ash contents (%) of studied diets.*

Diet	Protein (%)	Fat (%)	Moisture (%)	Ash (%)
Commercial feed	42.00	10.00	10.00	12
5% raw *Spirulina platensis* incorporated diet	42.9	9.75	13.76	11.55
10% raw *Spirulina platensis* incorporated diet	43.8	9.5	17.53	11.1
15% raw *Spirulina platensis* incorporated diet	44.7	9.25	21.29	10.56
20% raw *Spirulina platensis* incorporated diet	45.6	9	25.06	10.2
25% raw *Spirulina platensis* incorporated diet	46.5	8.75	28.82	9.75

3.2. Carotenoid Concentrations of Studied Diets

Increase of carotenoid contents in the diets were observed with the increase of raw *Spirulina platensis* percentage in the diet Table 2.

Table 2. *Carotenoid contents (mg/ml) of studied diets.*

Diet type	Carotenoid content (mg/ml)
Commercial feed	0.03
5% raw *Spirulina platensis* incorporated diet	0.06
10% raw *Spirulina platensis* incorporated diet	0.07
15% raw *Spirulina platensis* incorporated diet	0.07
20% raw *Spirulina platensis* incorporated diet	0.09
25% raw *Spirulina platensis* incorporated diet	0.10

3.3. Growth Performance of Guppy Fish

3.3.1. Mean Body Weight

The mean body weights of guppy fish fed with different diets at the end of study period (60 days) were shown in Figure 1. Significantly ($P<0.05$) high mean body weight (0.45 ± 0.09 g) was observed in guppy fish fed with 5% raw *Spirulina platensis* incorporated diet the lowest mean body weight (0.36 ± 0.13 g) was observed in guppy fish fed with the commercial diet

(Figure 1).

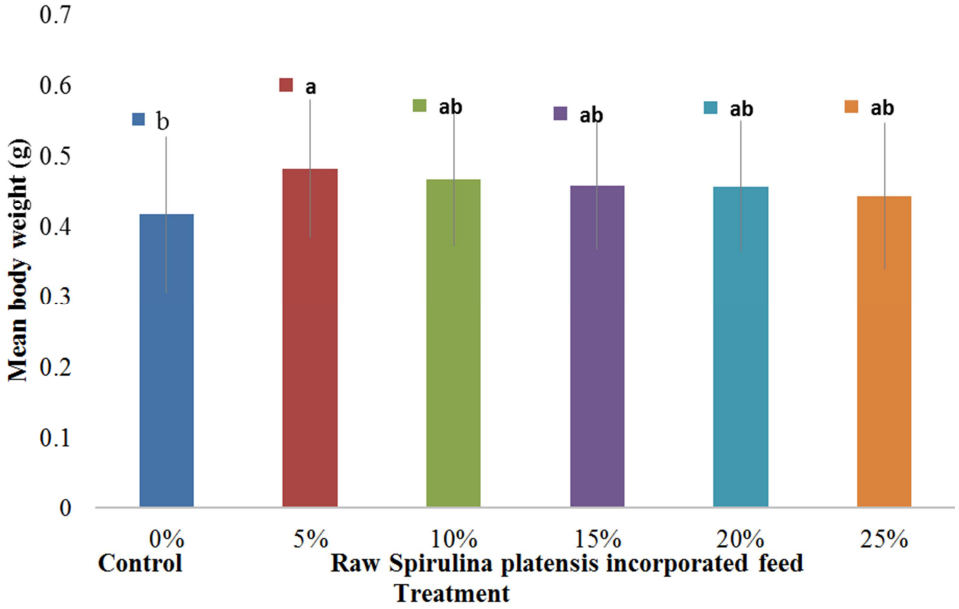

Figure 1. *Mean body weight of guppy fish over the study period (21-day old to 80-day old).*

*The treatments that are not significantly different shares the same letter

3.3.2. Mean Body Length

The mean body lengths of guppy fish fed with different diets at the end of study period (60 days) were shown in Figure 2. The lowest mean body length (27.65 ± 3.92 mm) was observed in guppy fish fed with the commercial feed. Significantly different ($P<0.05$) high mean body length (33.45 ± 3.21 mm) was observed in guppy fish fed with 5% raw *Spirulina platensis* incorporated feed after the study period.

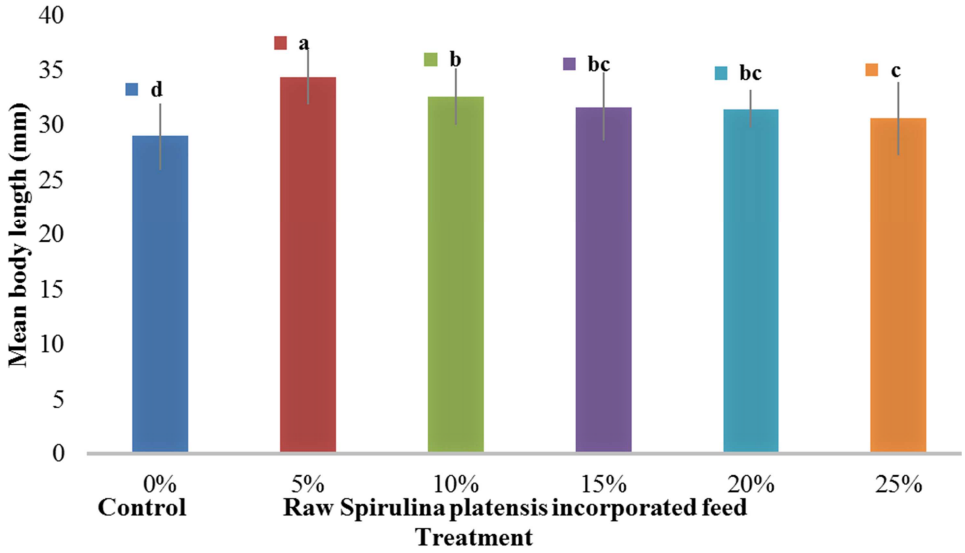

Figure 2. *Mean body length of guppy fish over the study period (21-day old to 80-day old).*

*The treatments that are not significantly different shares the same letter

3.3.3. Body Weight Gain of Fish

The mean body weights gains of guppy fish fed with different diets at the end of study period (60 days) were shown in Figure 3. The highest weight gain (0.37 ± 0.04 g) was recorded in guppy fed with 5% raw *Spirulina platensis* incorporated diet and the lowest body weight gain was (0.33 ± 0.05 g) in guppy fed with commercial feed. There were no significant differences ($P>0.05$) among different treatments (Figure 3).

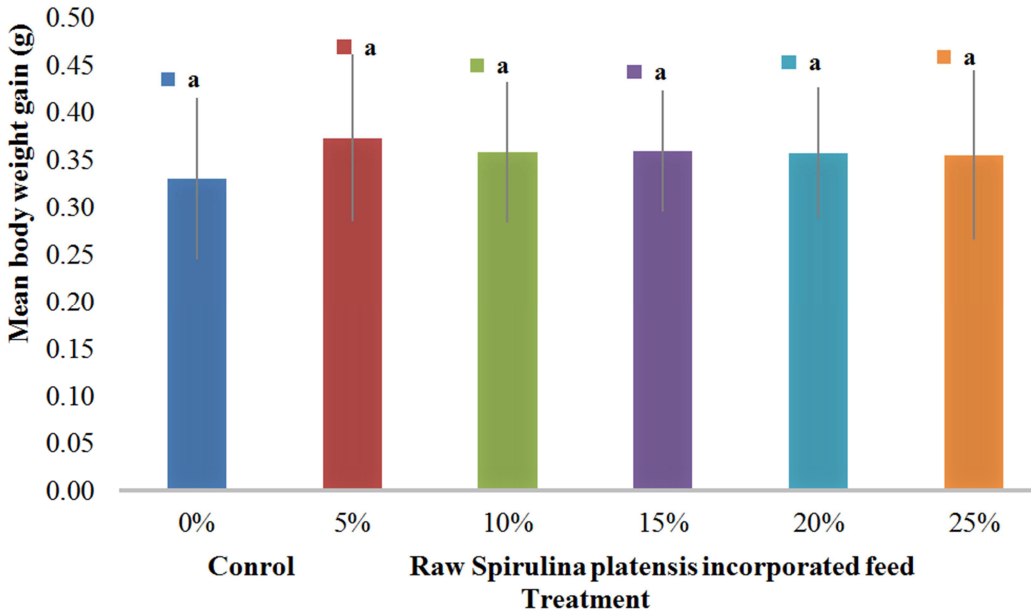

Figure 3. *Mean weight gain of guppy fish over the study period (21-day old to 80-day old).*

*The treatments that are not significantly different shares the same letter

3.3.4. Body Length Gain of Fish

The mean body length gains of guppy fish fed with different diets at the end of study period (60 days) were shown in Figure 4. The highest value for body length gain (16.44 ± 1.21 mm) was observed in guppy fish fed with 5% raw *Spirulina platensis* incorporated diet. The lowest length gain (11.25 ± 2.16 mm) was shown in guppy fish fed with the commercial diet. There were no significant differences (P>0.05) among the raw *Spirulina platensis* incorporated diets in terms of mean body length gain, except between the 25% *Spirulina platensis* incorporated diet and the control diet (Figure 4).

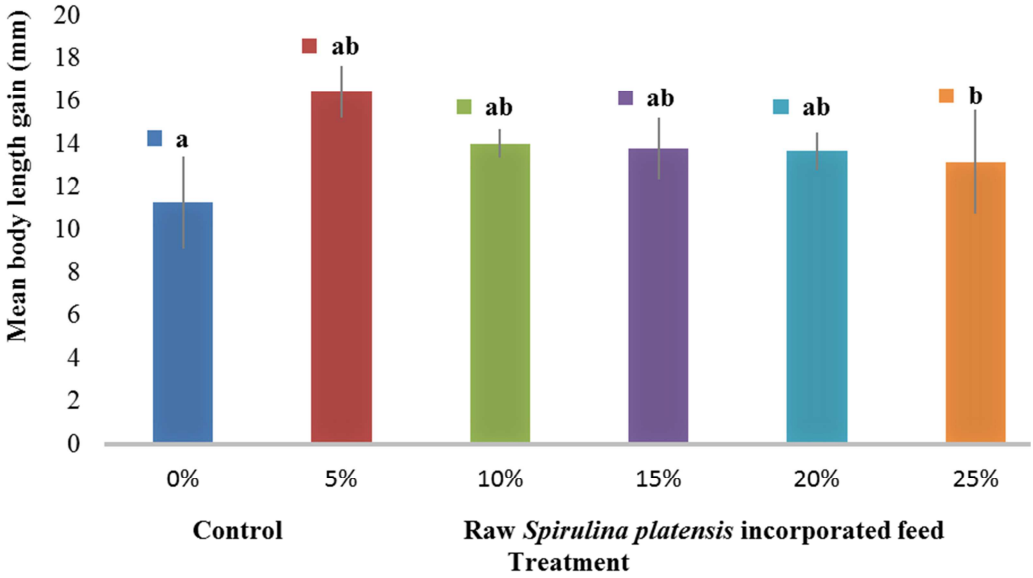

Figure 4. *Mean body length gain of guppy fish over the study period (21-day old to 80-day old).*

*The treatments that are not significantly different shares the same letter

3.3.5. Specific Growth Rate (SGR)

There was no significant difference (P>0.05) among the mean specific growth rate of fish due to different treatments. However, the highest value for the specific growth rate (0.04 ± 0.01 days^{-1}) was recorded for the fish fed with 5% raw *Spirulina platensis* incorporated diet (Figure 5).

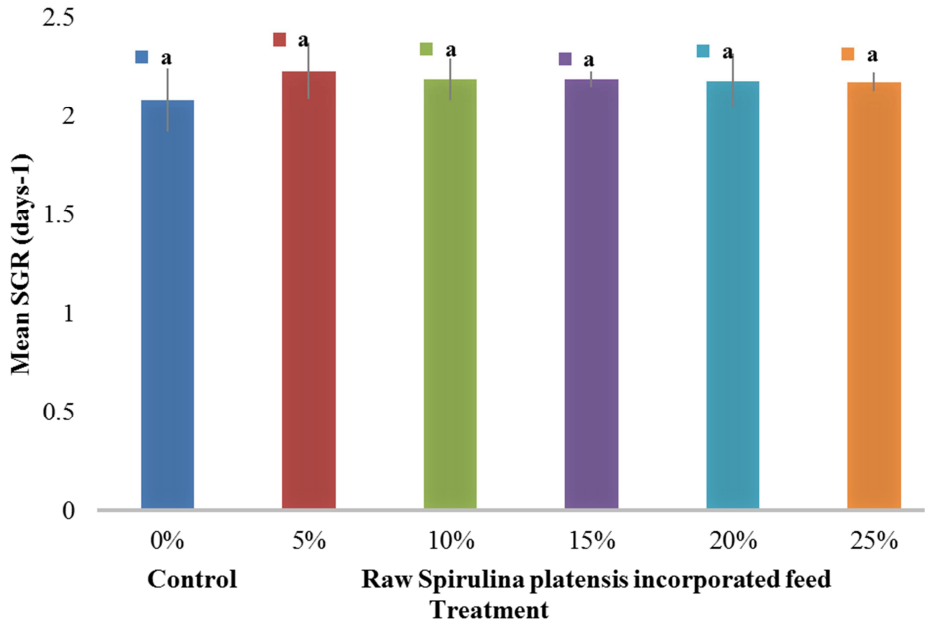

Figure 5. *Mean Specific growth rate of guppy fish over the study period (21-day old to 80-day old).*

*The treatments that are not significantly different shares the same letter

3.3.6. Feed Conversion Ratio (FCR)

The lowest feed conversion ratio was recorded as 1.43 ± 0.06 in guppy fish fed with 5% raw *Spirulina platensis* incorporated diet whereas the highest (2.06 ± 0.09) was observed in fish fed with commercial feed. There were no significant differences (P>0.05) among the *Spirulina platensis* incorporated treatment diets (Figure 6).

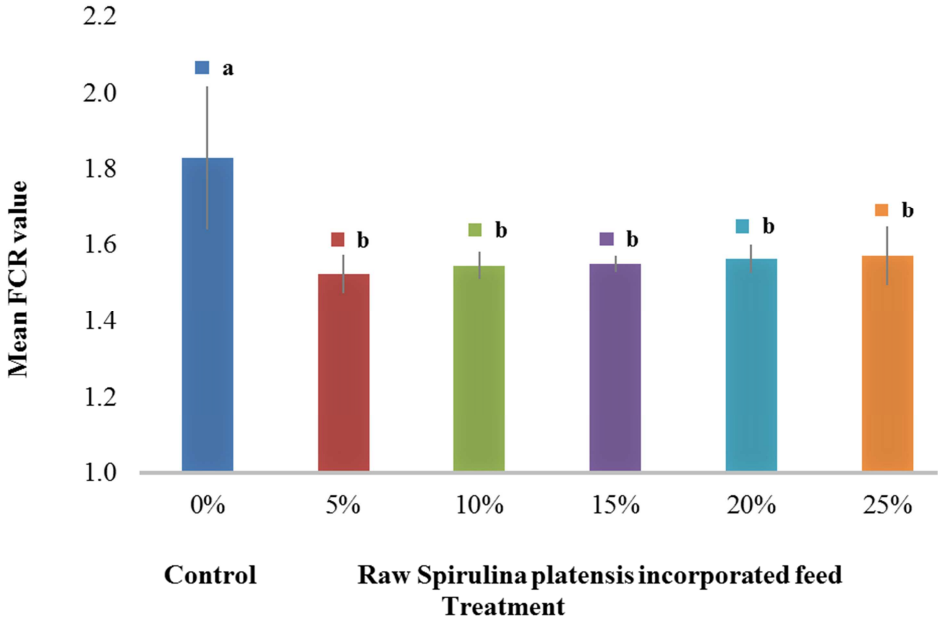

Figure 6. *Feed conversion ratio of guppy fish.*

*The treatments that are not significantly different shares the same letter

3.4. Body Colour Enhancement

The lowest mean of pixel value 110.94 ± 0.02 was shown by guppy fish fed with 25% raw *Spirulina platensis* incorporated diet whereas the highest mean of pixel value 119.9 ± 0.0 was recorded for the fish fed with the commercial feed. However, there were no significant differences (P>0.05) among the different *Spirulina platensis* incorporated diets (Figure 7). Means which have higher values represent the lowest colour intensities by imageJ software while the lower values represent the

higher colour intensities.

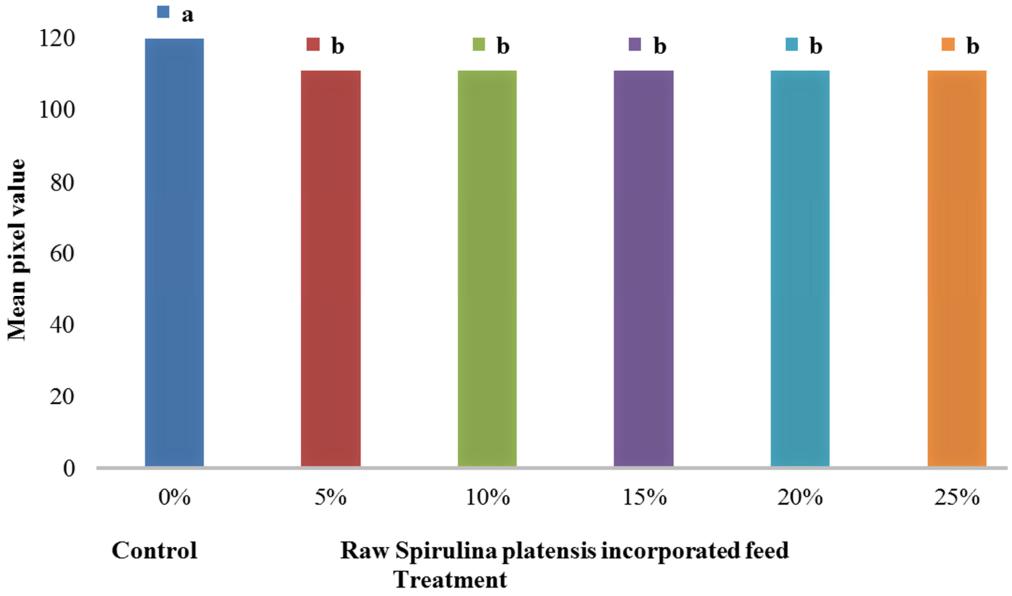

Figure 7. *Mean pixel values for body colour of 80-day old guppy fish.*

*The treatments that are not significantly different shares the same letter

3.5. Survival Rate

Survival rate was 100% in all treatments throughout the 60-day experimental period.

4. Discussion

Alternative feed supplements of plant origin in enhancing growth performances and colourations of ornamental fish to replace fish meal and other protein sources of animal origin which are of high cost are considered a priority in the growth of aquaculture industry [11-13]. Commercial importance *Spirulina platensis* due to its overall nutritional qualities and its use as a partial supplement or a complete replacement of the protein source was well documented [1]. In addition to the well-balanced major nutrients, *Spirulina platensis* contains essential amino acids, essential fatty acids, vitamins, minerals and pigments [2, 3]. In the present study also the increase of protein in the diet with the increase of percentage of *Spirulina platensis* in the diet is observed.

With the increase of the percentage of incorporating raw *Spirulina platensis*, the growth performances showed a declining trend. Similar results have been reported after a study conducted with *Spirulina platensis* dietary supplementation for tilapia fish [21]. Higher replacement levels did not produce further improvement in fish growth, but the values were numerically higher than those of the parrot fish fed the control diet [22]. Dietary replacement of *Spirulina platensis* can alter the body composition of the fish changing the protein and the lipid content of the tissues [14]. In addition to the high protein content, *Spirulina platensis* contain essential amino acids, gamma linolenic acid, beta carotene, phycocyanin pigments and vitamins and minerals in adequate quantities [23]. Therefore, *Spirulina platensis* can be directly used as a feed supplement without adding any other nutrient. Similar results have been reported by a previous study as, 5% supplementation of powdered *Spirulina platensis* resulted in higher body weight gain in *Girella punctata* (Gray) [24]. In addition, incorporation of powder form 5% *Spirulina platensis* meal gained significant enhancement of growth and feed utilization in one-year old red sea bream [14]. In general, *Spirulina platensis* incorporated diets showed better performances in the present study. Fish fed with commercial diet showed the lowest mean length and lowest mean body weight values in both Grow out I and Grow out II stages. Fish fed with the commercial diet showed the lowest mean body lengths in Grow out I (24.94 ± 33.99 mm) and in Grow out II (27.65 ± 3.92 mm). In terms of mean body weight and body length gain, raw *Spirulina platensis* incorporated diets have significantly higher body weight gain (0.25 ± 0.05 g) and length gain (13.14 ± 3.54 mm) in Grow out I. The lowest weight gain and length gain was observed in the fish fed with the commercial diet. In Grow out II, best weight gain (0.37 ± 0.04 g) and significantly high length gain (16.44 ± 1.21 g) showed in fish fed with 5% raw *Spirulina platensis* incorporated diet. *Spirulina platensis* has no cellulose cell wall and it helps in better digestion and adsorption that leads to better digestibility of protein and fat. Thus, dietary supplementation of *Spirulina platensis* leads to enhanced nutrient absorption [25].

Growth of guppy fish fed with a 5% raw *Spirulina platensis* incorporated diet showed the most favorable length by reaching total length up to 31.47 mm after 30 days (Grow out I) and up to 33.45 mm length after 60 days (Grow out II) with 100% survival rate. The highest length was observed in fish fed with 5% raw *Spirulina platensis* incorporated diet

with mean body weight (0.38 ± 0.06 g) in Grow out I and maximum average body weight (0.45 ± 0.09 g) in Grow out II. A higher specific growth rate (0.04 ± 0.00 days $^{-1}$) in guppy fish at Grow out I was observed in the fish fed with 5% raw *Spirulina platensis* incorporated feed. The lowest specific growth rate values in Grow out I (0.03 ± 0.00 days $^{-1}$) and the lowest in Grow out II (0.03 ± 0.01 days $^{-1}$) were observed for the commercial diet. The lowest feed conversion ratio (1.43 ± 0.06) was measured in guppy fish fed with 5% raw *Spirulina platensis* incorporated diet and the highest value (2.06 ± 0.09) of food conversion ratio was found for the commercial diet.

Dietary incorporation of *Spirulina platensis* does not affect the survival rates of Angel fish (*Pterophyllum scalare*) if the water quality parameters are maintained in optimum conditions [26]. In the present study also, the survival rates of fish were not affected by different diets. *Spirulina platensis* dried powder supplementation (40%) in the diet for guppy fish has a positive effect on the growth and feed conversion (1.090 ± 0.044), yet this study has not reported significant differences for mean final body weights of the studied fish [9]. In the present study, the dietary incorporation of lower parentages of raw *Spirulina platensis* demonstrated better growth performance.

Feeding tilapia with *Spirulina platensis* in the first period of larval development could accelerate their growth [27]. In the present study, all raw *Spirulina platensis* incorporated diet showed high rates of growth performance in Grow out I. The lesser improvement in later stage could be due to the reduction of protein requirement with the aging of fish. Use of raw *Spirulina platensis* dietary supplementation for juvenile tilapia has showed to be effective over the control diet used [28]. The present study also used raw *Spirulina platensis* for feed supplementation. Use of raw form is effective than using powder form as it is cost effective and favorable in keeping the original nutritional properties. Drying or heating can lead to destroy some vitamins like heat unstable nutrients.

In the present study, highest carotenoid content was observed in 25% raw *Spirulina platensis* incorporated diet. It could be due to high amount of carotenoid content in *Spirulina platensis*. As a pigmentation additive, *Spirulina platensis* has been used to enhance the colour of different fish species such as red tilapia, Mekong giant catfish, gold fish, prawn, and rainbow trout [20, 29-31]. Phycocynin (blue) and chlorophyll (green) pigments responsible for blue green colour of *Spirulina platensis* and other pigments such as xanthophylls, β-carotene, echinenone, phycocyanin, zeaxanthin and cryptoxanthin make this micro algae ideal to use as a colour enhancement agent for ornamental fish species [4, 5]. Increase of the levels of *Spirulina platensis* in the diet was led to colour enhancement in rain bow trout flesh. The highest levels of fillet pigmentation and carotenoid deposition were found in highest *Spirulina platensis* powder added diet [20]. In this study also 25% raw *Spirulina platensis* added diet showed the best result in both grow out stages. For the whole experimental period, 25% raw *Spirulina platensis* added diet showed the highest mean, but overall result showed nearly similar mean values. Similar results have been reported for African sharp tooth catfish, striped jack, sweet smelt fish and Mekong giant catfish with the *Spirulina platensis* addition to the diets by previous studies [30, 32-34]. Use of 8% *Spirulina platensis* as a dietary supplementation led to give improved coloration in sword tail fish *(Xiphophorus hellerii*) [35]. However, the present study did not show any significant differences among the colouration of fish with the incorporated different percentages of *Spirulina platensis* levels.

5. Conclusion

As per the results gained in this study, all the raw *Spirulina platensis* incorporated diets showed better growth performance in terms of mean body weight, mean body length, specific growth rate and mean length gain in guppy fish over the control diet used. All the raw *Spirulina platensis* incorporated diets showed enhanced body colour in fish with compared to the fish fed with the commercial diet although not significantly different.

References

[1] Mohanty, D., & Samanta, L. (2018). Dietary supplementation of *Spirulina ameliorates* iron-induced oxidative stress in Indian knife fish Notopterus Notopterus. Environmental toxicology and pharmacology, 61, 71-78.

[2] Simpore, J., Kabore, F., Zongo, F., Dansou, D., Bere, A., Pignatelli, S.,... & Musumeci, S. (2006). Nutrition rehabilitation of undernourished children utilizing Spiruline and Misola. Nutrition journal, 5 (1), 3.

[3] Khafaga, A. F., & El-Sayed, Y. S. (2018). *Spirulina ameliorates* methotrexate hepatotoxicity via antioxidant, immune stimulation, and proinflammatory cytokines and apoptotic proteins modulation. Life sciences, 196, 9-17.

[4] Abdel-Daim, M. M. (2014). Pharmacodynamic interaction of *Spirulina platensis* with erythromycin in Egyptian Baladi bucks (*Capra hircus*). Small Ruminant Research, 120 (2-3), 234-241.

[5] Adel, M., Yeganeh, S., Dadar, M., Sakai, M., & Dawood, M. A. (2016). Effects of dietary *Spirulina platensis* on growth performance, humoral and mucosal immune responses and disease resistance in juvenile great sturgeon (*Huso huso Linnaeus,* 1754). Fish & shellfish immunology, 56, 436-444.

[6] Raghavan, R., Dahanukar, N., Tlusty, M. F., Rhyne, A. L., Kumar, K. K., Molur, S., & Rosser, A. M. (2013). Uncovering an obscure trade: threatened freshwater fishes and the aquarium pet markets. Biological Conservation, 164, 158-169.

[7] Marudhupandi, T., Kumar, T. T. A., Prakash, S., Balamurugan, J., & Dhayanithi, N. B. (2017). *Vibrio parahaemolyticus* a causative bacterium for tail rot disease in ornamental fish, *Amphiprion sebae*. Aquaculture Reports, 8, 39-44.

[8] Ghosh, A., Mahapatra, B. K., & Datta, N. C. (2003). Ornamental fish farming-successful small scale aqua business in India. Aquaculture Asia, 8 (3), 14-16.

[9] Dernekbasi, S., Unal, H., Karayucel, I., & Aral, O. (2010). Effect of dietary supplementation of different rates of spirulina (*Spirulina platensis*) on growth and feed conversion in Guppy (*Poecilia reticulata* Peters, 1860). Journal of Animal and Veterinary Advances, 9 (9), 1395-1399.

[10] Görelşahin, S., Yanar, M., & Kumlu, M. (2018). The effects of stocking density, Tubifex feeding and monosex culture on growth performance of guppy (*Poecilia reticulata*) in a closed indoor recirculation system. Aquaculture, 493, 153-157.

[11] Macias-Sancho, J., Poersch, L. H., Bauer, W., Romano, L. A., Wasielesky, W., & Tesser, M. B. (2014). Fishmeal substitution with *Arthrospira* (*Spirulina platensis*) in a practical diet for *Litopenaeus vannamei*: effects on growth and immunological parameters. Aquaculture, 426, 120-125.

[12] Cao, S., Zhang, P., Zou, T., Fei, S., Han, D., Jin, J.,... & Xie, S. (2018). Replacement of fishmeal by *spirulina Arthrospira platensis* affects growth, immune related-gene expression in gibel carp (*Carassius auratus* gibelio var. CAS III), and its challenge against Aeromonas hydrophila infection. Fish & shellfish immunology, 79, 265-273.

[13] Raji, A. A., Alaba, P. A., Yusuf, I., Bakar, N. H. A., Taufek, N. H. M., Muin, H.,... & Razak, S. A. (2018). Fishmeal replacement with *Spirulina Platensis* and *Chlorella vulgaris* in African catfish (*Clarias gariepinus*) diet: Effect on antioxidant enzyme activities and haematological parameters. Research in veterinary science.

[14] Mustafa, M. G., Takeda, T., Umino, T., Wakamatsu, S., & Nakagawa, H. (1994). Effects of *Ascophyllum* and *Spirulina* meal as feed additives on growth performance and feed utilization of red sea bream, Pagrus major. Journal of the Faculty of Applied Biological Science-Hiroshima University (Japan).

[15] Watanuki, H., Ota, K., Tassakka, A. C. M. A., Kato, T., & Sakai, M. (2006). Immunostimulant effects of dietary *Spirulina platensis* on carp, *Cyprinus carpio*. Aquaculture, 258 (1-4), 157-163.

[16] Abdelkhalek, N. K., Ghazy, E. W., & Abdel-Daim, M. M. (2015). Pharmacodynamic interaction of *Spirulina platensis* and deltamethrin in freshwater fish Nile tilapia, *Oreochromis niloticus*: impact on lipid peroxidation and oxidative stress. Environmental Science and Pollution Research, 22 (4), 3023-3031.

[17] Pandey, J. P., Pathak, N., & Tiwari, A. (2010). Standardization of pH and light intensity for the biomass production of *Spirulina platensis*. Journal of Algal Biomass Utilization, 1 (2), 93-102.

[18] Gonzales, J., & John, M. (2012). Preliminary evaluation on the effects of feeds on the growth and early reproductive performance of zebrafish (*Danio rerio*). Journal of the American Association for Laboratory Animal Science, 51 (4), 412-417.

[19] Hartig, S. M. (2013). Basic image analysis and manipulation in ImageJ. Current protocols in molecular biology, 102 (1), 14-15.

[20] Teimouri, M., Amirkolaie, A. K., & Yeganeh, S. (2013). The effects of *Spirulina platensis* meal as a feed supplement on growth performance and pigmentation of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 396, 14-19.

[21] Olvera‐Novoa, M. A., Domínguez‐Cen, L. J., Olivera‐Castillo, L., & Martínez‐Palacios, C. A. (1998). Effect of the use of the microalga *Spirulina maxima* as fish meal replacement in diets for tilapia, *Oreochromis mossambicus* (Peters), fry. Aquaculture research, 29 (10), 709-715.

[22] Kim, S. S., Rahimnejad, S., Kim, K. W., & Lee, K. J. (2013). Partial replacement of fish meal with *Spirulina pacifica* in diets for parrot fish (*Oplegnathus fasciatus*). Turkish Journal of Fisheries and Aquatic Sciences, 13 (2).

[23] Nandeesha, M. C., Gangadhar, B., Varghese, T. J., & Keshavanath, P. (1998). Effect of feeding *Spirulina platensis* on the growth, proximate composition and organoleptic quality of common carp, *Cyprinus carpio* L. Aquaculture Research, 29 (5), 305-312.

[24] Nakazoe, J. I. (1986). Effect of supplementation of alga or lipids to the diets on the growth and body composition of nibbler *Girella punctata* Grey. Bull Tokai Reg Fish Res Lab, 120, 43-51.

[25] Becker, E. W., & Venkataraman, L. V. (1984). Production and utilization of the blue-green alga Spirulina in India. Biomass, 4 (2), 105-125.

[26] Moosavi, B. J., & Montajami, S. (2013). Assessment the Effect of *Spirulina platensis* as Supplemental Feed on Growth Performance and Survival Rate in Angel Fish (*Pterophyllum scalare*). Journal of Fisheries International, 8 (3), 74-77.

[27] Lu, J., Yoshizaki, G., Sakai, K., & Takeuchi, T. (2002). Acceptability of raw *Spirulina platensis* by larval tilapia *Oreochromis niloticus*. Fisheries science, 68 (1), 51-58.

[28] Takeuchi, T., Lu, J. U. N., Yoshizaki, G., & Satoh, S. (2002). Effect on the growth and body composition of juvenile tilapia *Oreochromis niloticus*. Fisheries Science, 68 (1), 34-40.

[29] Lu, J., Takeuchi, T., & Ogawa, H. (2003). Flesh quality of tilapia *Oreochromis niloticus* fed solely on raw Spirulina. Fisheries science, 69 (3), 529-534.

[30] Tongsiri, S., Mang-Amphan, K., & Peerapornpisal, Y. (2010). Effect of replacing fishmeal with *Spirulina* on growth, carcass composition and pigment of the Mekong giant catfish. Asian Journal of Agricultural Sciences, 2 (3), 106-110.

[31] Nur-E-Borhan, S. A. (1993). Pigmentation of Cultured Black Tiger Prawn by Feeding with a *Spirulina*-Supplemented Die, Bulletin of the Japanese Society for the Science of Fish, 59 (1), 165-169.

[32] Promya, J., & Chitmanat, C. (2011). The effects of *Spirulina platensis* and Cladophora algae on the growth performance, meat quality and immunity stimulating capacity of the African sharptooth catfish (Clarias gariepinus). International Journal of agriculture and Biology, 13 (1).

[33] Okada, S., Yamaguchi, M., Yamaguchi, K. & Watanabe, T. (1991). Pigmentation of Cultured Striped Jack Reared on Diets Supplemented with the Blue-Green Alga *Spirulina maxima*., 57 (7), 1403- 1406.

[34] Mori, T., Muranaka, T., Miki, W., Yamaguchi, K., Konosu, S., & Watanabe, T. (1987). Pigmentation of cultured sweet smelt fed diets supplemented with a blue-green algae *Spirulina maxima*. Nippon Suisan Gakkaishi, 53 (3), 433-438.

[35] James, R., Sampath, K., Thangarathinam, R., & Vasudevan, I. (2006). Effect of dietary *spirulina* level on growth, fertility, coloration and leucocyte count in red swordtail, *Xiphophorus helleri*. Isr. J. Aquac. Bamidgeh, 58 (2): 97-104.