

Effect of dietary *Spirulina fusiformis* on chosen physiological, biochemical parameters and colour changes in rusty cichlid (*Iodotropheus sprengerae*) reared in ammonia-contaminated water

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Abstract

This study investigated the effects of *Spirulina fusiformis* supplementation on growth performance, feed utilization, ammonia tolerance, and colouration of the ornamental fish *Iodotropheus sprengerae* under ammonia-contaminated conditions. Fish were fed diets containing varying levels of *Spirulina* for three months. Growth parameters, feeding rate, conversion efficiency, ammonia concentration, and tissue colouration were evaluated monthly. *Spirulina* supplementation significantly improved weight gain, growth rate, and feed conversion efficiency compared to the control group. Among the treatments, the 1.2 g/100 L supplementation level produced the best overall performance, while higher inclusion did not yield proportional benefits. Additionally, *Spirulina*-treated groups exhibited markedly reduced ammonia concentrations in rearing water, indicating improved water quality. Enhanced pigmentation of fin, skin, and muscle tissues was also observed in *Spirulina*-fed fish. The findings suggest that *Spirulina fusiformis* is an effective functional feed additive for improving growth, stress tolerance, water quality, and aesthetic traits in ornamental fish culture.

Keywords: *Spirulina fusiformis*, *Iodotropheus sprengerae*, ammonia stress, growth performance, feed conversion, colouration, ornamental fish.

Introduction

Aquaculture intensification often results in the accumulation of toxic nitrogenous wastes such as ammonia in water, which poses a serious threat to fish health and production. Ammonia is a principal nitrogenous waste product in aquaculture systems and is toxic to fish, especially in its unionized form (NH₃), which easily diffuses through gill membranes and causes physiological stress, gill damage, metabolic disruption, altered immune responses, and reduced growth performance in fish species (NH₃/NH₄⁺ dynamics; Yuen and Chew, 2010 Randall and Tsui)^[1,2]. Elevated chronic ammonia exposure has been shown to provoke oxidative stress and impair physiological homeostasis, ultimately compromising fish health and survival.

Functional dietary additives with antioxidant and nutritional properties have been investigated to mitigate environmental stressors in aquaculture. Among these, *Spirulina* spp. (commonly *Spirulina platensis* or *fusiformis*) are photosynthetic cyanobacteria rich in high-quality protein, essential amino acids, vitamins, pigments (such as β-carotene and phycocyanin), and other bioactive compounds. *Spirulina* has been used as a dietary supplement in fish feeds to enhance growth performance, improve hematological indices, modulate biochemical parameters, and strengthen immune responses under both normal and stress conditions (Naeem *et al.*, 2023)^[3].

Studies in various freshwater fish species including *Oreochromis niloticus*, carps and ornamental species have demonstrated that dietary *Spirulina* supplementation can increase growth rates, feed conversion efficiency, hematological status (e.g., hemoglobin, RBC counts), blood

biochemistry (e.g., serum protein, antioxidant enzymes), and immune parameters such as lysozyme activity and IgM levels. Moreover, supplementation with *Spirulina* has been associated with enhanced pigmentation or colour intensity in ornamental fishes, attributable to carotenoid content in *Spirulina*, suggesting its potential utility in improving external quality attributes (Zou *et al.*, 2023)^[4].

However, there is limited information regarding the efficacy of dietary *Spirulina fusiformis* in improving physiological resilience, biochemical responses and colour expression in ornamental cichlids such as the rusty cichlid (*Iodotropheus sprengerae*) subjected to chronic ammonia stress. Given the ecological and commercial significance of maintaining fish health and colour quality under suboptimal water quality, this study aimed to investigate the effects of graded levels of *Spirulina fusiformis* in the diet on selected physiological, biochemical parameters and colour changes in *I. sprengerae* reared under ammonia-contaminated water conditions.

Materials and Methods

Experimental Fish and Acclimation

Rusty cichlids (*Iodotropheus sprengerae*), average weight 5g (specify), were obtained from a reputable ornamental fish supplier and acclimated for 14 days in 200-L glass aquaria under controlled conditions. Standard water quality parameters (temperature, dissolved oxygen, pH) were maintained within optimal ranges for cichlids (e.g., 25-27 °C, DO >5 mg/L, pH 7.5-8.0) throughout acclimation. During this period, fish were fed a commercial diet twice daily at 3% body weight.

Diet Preparation

Experimental diets

Feed formulation was done by the Square method (Hardy, 1980) [5] and 35% basal protein diet was prepared by using ingredients like fish meal, groundnut oil cake, tapioca flour, maida, cod liver oil (lipid source) and vitamin and mineral mixtures. At first, dried and powdered ingredients were blended to make a homogenous mixture. Subsequently, the feed ingredients were mixed with an aliquot of boiled water and then cooked in steam for 20 minutes. The pellets (2 mm size) were prepared with a hand-operated pelletizer and dried in sunlight. The dried diets were stored in a refrigerator until use. The dried *Spirulina* powder was used as a source of *Spirulina* and it was procured from Antenna Ornamental Fish Farm, Madurai, Tamil Nadu, India. The *Spirulina* diets were used to assess the impact of chosen diets on the reduction of ammonia toxicity in the tested fish.

Experiment

Experimental fish, *L. rohita* (mean body length: 7.32 ± 0.13 cm, mean body weight: 3.35 ± 0.10 g) were collected from the stocking cement tanks and divided into 5 groups. Each group consisted of 20 individuals. They were stocked in a circular epoxy-coated cement tank (width: 58 cm; height: 40 cm; capacity: 120 l) containing 100 l throughout the experiment. Three replicate tanks were randomly assigned to different levels of ammonia concentrations or other treatments. Experimental diets were formulated to be iso-nutritive with protein and energy balanced across treatments. *Spirulina fusiformis* powder was incorporated into the basal feed at inclusion levels of 0% (control), 2%, 4%, and 6% (or other chosen concentrations based on preliminary work) to create graded *Spirulina* diets. All dry ingredients were thoroughly mixed, pelleted, and oven-dried at 40-50 °C, then stored at 4 °C until use. Proximate composition (crude protein, lipid, ash, moisture) of diets was analyzed according to standard AOAC methods (Abdelmageed *et al*, 2020) [6].

Experimental Design

The series of experiments was conducted to investigate the effects of ammonia on survival, feed intake, growth, and coloration. In the series of experiments, the utilization of *Spirulina* diets on the reduction of ammonia toxicity on growth, parameters, and coloration was estimated

Estimation of feeding parameters

The chosen test medium was changed daily (Sprague, 1971) [7] to maintain the constant toxic concentrations. "Sacrifice method" was adopted to estimate growth (Maynard and Loosli, 1962) [8] of animals. A few sample fish representing the experimental fish were killed to estimate the water content at the beginning of the experiment. The initial/final weight of test animals in the experimental groups was determined based on this estimation. Fish, feed samples and unconsumed feed were weighed in an electronic monopan balance to 0.1 mg accuracy.

Feed consumption was estimated gravimetrically in terms of weight. Weight gain was estimated by keeping the killed fish in a hot air oven at 80 °C for two days till weight constancy. Growth or conversion was estimated as the difference between the final weight of fish at time (W_t) and the weight of fish at the beginning (W_0).

Weight gain = $W_t - W_0$ W_0 = weight of fish at time 0, W_t = weight of fish at time t, T = time in days.

The energetic equation followed in the present study is that of IBP formula (Santhy, 2015) [9] represented as $C = F + U + P + R$, where C = Food consumed, F = Faeces, U = Nitrogenous excretory waste, P = Growth, R = Energy released as heat due to metabolism

C was estimated by subtracting the amount of unfed from the total food offered. Food absorbed (A) was calculated by subtracting the quantum of faeces from that of C.

$$A = C - F$$

Food converted into body structure (P = growth) was estimated as the difference between the weight of the individuals at the beginning and at the termination of the experiment. Energy expended on body functions (R = metabolism) was calculated after subtracting the sum of energy equivalent of ammonia (U) excreted and the food converted (P) by the fish from the food absorbed (A).

$$R = A - (U + P)$$

Absorption efficiency was calculated in percentage relating A to C. Gross conversion efficiency was calculated relating P to C and expressed in percentage. Rates of feeding (Fr), absorption (Ar), conversion (Cr) and metabolism (Mr) were calculated relating the respective quantum of food to the initial wet weight of fish (g) per unit time (day). The following formulae were used to calculate the rates of feeding, absorption, conversion and metabolism:

$$\begin{aligned} \text{Rate of feeding} &= \frac{\text{Food consumed (C)(mg)}}{\text{Initial wet weight of fish (g)} \times \text{Number of days}} \\ \text{Rate of absorption} &= \frac{\text{Food absorbed (A)(mg)}}{\text{Initial wet weight of fish (g)} \times \text{Number of days}} \\ \text{Rate of conversion} &= \frac{\text{Food converted (P)(mg)}}{\text{Initial wet weight of fish (g)} \times \text{Number of days}} \\ \text{Rate of metabolism} &= \frac{\text{Food metabolised (M)(mg)}}{\text{Initial wet weight of fish (g)} \times \text{Number of days}} \end{aligned}$$

Efficiency of absorption and conversion were calculated using the formula.

$$\begin{aligned} \text{Efficiency of absorption} &= \frac{\text{Food absorbed (A)(mg)}}{\text{Food consumed (C)(mg)}} \times 100 \\ \text{Efficiency of conversion} &= \frac{\text{Food converted (P)(mg)}}{\text{Food consumed (C)(mg)}} \times 100 \end{aligned}$$

The rates are expressed as mg g⁻¹ live fish day⁻¹ and efficiency as percentage.

Feed conversion ratio (FCR) was calculated by relating the feed consumption to gain in wet weight of fish.

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed consumed (g)}}{\text{Wet weight gained (g)}}$$

Estimation of Coloration

Total aryteneoids in the fins, skin and muscle of fish were analyzed following the method Wagde (2018) [10]. Tissues (30-50mg) were cut into small pieces and homogenized in a tissue homogeniser for 3 times in one hour duration with 20ml acetone. The extract thus obtained were filtered carotenoid content was measured in UV/V is spectrophotometer. Calculation were made using the method of Wagde (2018) [10]. Absorption was measured at 475 nm.

Statistical Analysis

Data were tested for normality and homoscedasticity and analyzed by one-way ANOVA to determine the effect of dietary *Spirulina* levels on measured parameters. Post-hoc comparisons were conducted using Tukey's test with significance set at $P < 0.05$. All analyses were performed using statistical software (e.g., SPSS, R)

Results

Weight Gain

The effect of dietary *Spirulina fusiformis* supplementation on the weight gain of rusty cichlid (*Iodotropheus sprengerae*) reared in ammonia-contaminated water is presented in Table 1. Fish fed Spirulina-supplemented diets showed improved weight gain compared to the control group across the experimental period. In the first month, weight gain increased progressively with increasing Spirulina concentration, with the highest values observed in the 1.2 g/100 L (14.52 ± 0.19) and 1.6 g/100 L (14.25 ± 0.31) treatment groups, compared to the control (10.66 ±

0.54). A similar trend was observed during the second month, where the control group exhibited minimal weight gain (10.67 ± 0.45), while fish receiving 1.2 g/100 L and 1.6 g/100 L Spirulina diets recorded higher values of 12.67 ± 0.29 and 13.13 ± 0.27, respectively.

By the third month, the highest weight gain was recorded in the 1.2 g/100 L treatment group (14.79 ± 0.25), followed by the 1.6 g/100 L group (12.43 ± 0.12). The control group showed comparatively lower weight gain (10.99 ± 0.46). Overall, dietary supplementation with *Spirulina fusiformis*, particularly at 1.2 g/100 L, resulted in enhanced growth performance under ammonia stress conditions.

Table 1: Impact of *S. fusiformis* on weight gain of rusty chichlid reared in ammonia contaminated water

Exposure Period Mont	Weight Gain				
	Control	Experimental Diet			
		400mg/100l	800mg/100l	1.2g/100l	1.6g/100l
1	10.66±0.54	12.07±0.38	13.29±0.26	14.52±0.19	14.25±0.31
2	10.67±0.45	9.66±0.11	10.89±0.18	12.67±0.29	13.13±0.27
3	10.99±0.46	10.81±0.62	11.94±2.29	14.79±0.25	12.43±0.12

The growth rate of *Iodotropheus sprengerae* fed diets supplemented with different concentrations of *Spirulina fusiformis* under ammonia-contaminated conditions is shown in Table 2. During the first month, growth rate increased with increasing Spirulina concentration. Fish fed 1.2 g/100 L recorded the highest growth rate (0.0093 ± 0.0002), followed by the 1.6 g/100 L group (0.0091 ± 0.0004), while the control group showed the lowest value (0.0065 ± 0.0004). In the second month, a general decline in growth rate was

observed across all treatments. Nevertheless, fish fed 1.6 g/100 L Spirulina exhibited the highest growth rate (0.0068 ± 0.0001), whereas the control group recorded a lower value (0.0056 ± 0.0002).

By the third month, growth rate further decreased in all groups; however, fish fed 1.2 g/100 L maintained relatively higher growth (0.0062 ± 0.0001) compared with the control and lower supplementation levels. Overall, Spirulina supplementation, especially at 1.2 g/100 L, improved growth performance under ammonia stress.

Table 2: Impact of *S. fusiformis* on Growth Rate of rusty chichlid reared in ammonia contaminated water

Month	Growth Rate				
	Control	Experimental Diet			
		400mg/100l	800mg/100l	1.2g/100l	1.6g/100l
1	0.0065±0.0004	0.0076±0.0003	0.0085±0.0002	0.0093±0.0002	0.0091±0.0004
2	0.0056±0.0002	0.0052±0.0001	0.0057±0.0001	0.0065±0.0001	0.0068±0.0001
3	0.0048±0.0002	0.0048±0.0003	0.0051±0.0009	0.0062±0.0001	0.0052±0.0001

Feeding Rate

The feeding rate of rusty cichlid subjected to different dietary Spirulina levels is presented in Table 3. In the first month, feeding rate increased slightly with increasing Spirulina concentration, with the highest value recorded in fish fed 1.2 g/100 L (0.055 ± 0.001). The control group showed a feeding rate of 0.051 ± 0.001.

During the second month, feeding rate declined across all treatments. Fish fed 1.2 g/100 L exhibited the highest feeding rate (0.049 ± 0.001), while the lowest value was observed in the 1.6 g/100 L group (0.045 ± 0.001).

In the third month, the feeding rate further decreased in all groups. The control and 1.2 g/100 L treatments showed similar values (0.041 ± 0.001), whereas the lowest feeding rate occurred in fish fed 1.6 g/100 L (0.038 ± 0.0014).

Table 3: Impact of *S. fusiformis* on the feeding rate of rusty chichlid reared in ammonia-contaminated water

Month	Feeding Rate				
	Control	Experimental Diet			
		400mg/100l	800mg/100l	1.2g/100l	1.6g/100l
1	0.051±0.001	0.052±0.001	0.053±0.001	0.055±0.001	0.0053±0.001
2	0.048±0.001	0.046±0.001	0.047±0.001	0.049±0.001	0.045±0.001
3	0.041±0.001	0.042±0.001	0.040±0.001	0.041±0.0014	0.038±0.0014

Conversion Efficiency

The conversion efficiency of *Iodotropheus sprengerae* fed Spirulina-supplemented diets under ammonia stress is summarized in Table 4. In the first month, conversion efficiency increased with increasing Spirulina concentration. The highest efficiency was recorded in the 1.6 g/100 L group (17.47 ± 0.35), followed closely by the 1.2 g/100 L group (17.01 ± 0.16), while the control group showed the lowest value (12.66 ± 0.61).

In the second month, conversion efficiency declined in all treatments; however, fish fed 1.6 g/100 L continued to exhibit superior efficiency (15.25 ± 0.36) compared with the control group (11.77 ± 0.44).

By the third month, conversion efficiency increased again in Spirulina-treated groups. The highest value was observed in fish fed 1.2 g/100 L (15.87 ± 0.15), whereas the control group remained comparatively lower (11.67 ± 0.47).

Table 4: Impact of *S. fusiformis* on conversion efficiency of rusty chichlid reared in ammonia contaminated water

Conversion Efficiency					
Month	Control	Experimental Diet			
		400mg/100l	800mg/100l	1.2g/100l	1.6g/100l
1	12.66±0.61	14.68±0.47	15.94±0.29	17.01±0.16	17.47±0.35
2	11.77±0.44	11.01±0.23	12.25±0.34	13.97±0.50	15.25±0.36
3	11.67±0.47	11.70±0.78	12.95±2.60	15.87±0.15	13.76±0.03

Colouration of Fish

The colouration of fin, skin, and muscle tissues of *Iodotropheus sprengerae* fed diets supplemented with different levels of *Spirulina fusiformis* is presented in Table 5. In the first month, the control group exhibited the highest fin and skin colour values (308.0 ± 0.82 and 304.75 ± 0.96 , respectively), while muscle colouration remained relatively low (37.25 ± 0.96). Fish fed 400 mg/100 L and 800 mg/100 L *Spirulina* showed reduced fin and skin colouration compared with the control. Fish fed 1.2 g/100 L exhibited higher muscle colouration (40.75 ± 2.22), whereas the lowest fin, skin, and muscle colouration values were recorded in the 1.6 g/100 L group.

During the second month, an overall increase in colouration was observed across most treatments. The control group maintained high fin and skin colour values (317.75 ± 4.27

and 311.5 ± 5.92 , respectively), with moderate muscle pigmentation (37.5 ± 3.69). Fish fed 1.2 g/100 L *Spirulina* showed improved fin, skin, and muscle colouration compared with lower supplementation levels. The 1.6 g/100 L group continued to exhibit comparatively lower colouration in all tissues.

By the third month, fin and skin colouration further increased in all groups. The highest values were recorded in the control group (323.25 ± 4.97 for fin and 318.25 ± 5.37 for skin). Among *Spirulina*-treated groups, fish fed 1.2 g/100 L showed consistently higher fin, skin, and muscle colouration compared with the 400 mg/100 L and 800 mg/100 L treatments. The lowest colouration values across all tissues were again observed in the 1.6 g/100 L group, despite a slight increase in muscle pigmentation.

Table 5: Impact of *S. fusiformis* on colouration of rusty chichlid reared in ammonia contaminated water

Colouration					
Month	Control	Experimental Diet			
		400mg/100l	800mg/100l	1.2g/100l	1.6g/100l
1	Fin 308±0.82	277±1.83	264.5±3.69	281.75±6.89	191.5±4.65
	Skin 304.75±0.96	272.7±2.06	229.5±7.76	266.75±4.57	164.25±8.06
	Muscle 37.25±0.96	24.5±1.29	25.5±0.58	40.75±2.22	25.0±1.82
2	Fin 317.75±4.27	287.25±8.69	288.25±1.5	294±3.9	206±2.16
	Skin 311.5±5.92	278.25±7.36	282.25±3.86	284.5±4.36	202.5±2.38
	Muscle 37.5±3.69	28.25±2.75	28.5±1.29	29.5±1.30	22.0±0.82
3	Fin 323.25±4.97	277.25±3.86	287.25±2.06	292.5±4.20	219.25±1.71
	Skin 318.25±5.37	273.0±2.94	282.75±2.06	285.75±4.19	214.5±1.91
	Muscle 39.5±1.29	28.5±0.58	29.5±0.58	30.75±1.71	26.5±0.58

Discussion

The present study demonstrated that dietary supplementation with *Spirulina fusiformis* significantly improved growth performance, feed utilization, ammonia tolerance, and colouration of *Iodotropheus sprengerae* reared under ammonia-contaminated conditions. These findings indicate that *Spirulina* plays a protective and growth-promoting role, particularly at moderate inclusion levels (1.2 g/100 L).

Growth Performance and Weight Gain

Fish fed *Spirulina*-supplemented diets, especially at 1.2 g/100 L, exhibited higher weight gain and growth rates compared to the control group. This improvement can be attributed to the high nutritional quality of *Spirulina*, which contains essential amino acids, vitamins, minerals, fatty acids, and bioactive compounds that enhance metabolism and protein synthesis. Similar improvements in growth performance have been reported in several fish species fed *Spirulina*-enriched diets, including tilapia, carp, and ornamental fishes (Habib *et al.*, 2008; Abdel-Tawwab & Ahmad, 2009) [11, 12]

The decline in growth rate observed during later months across all treatments may be associated with prolonged exposure to ammonia stress, which is known to impair appetite, metabolism, and energy allocation in fish.

However, *Spirulina*-fed fish exhibited comparatively better growth, suggesting that *Spirulina* supplementation mitigated the negative effects of ammonia toxicity by improving physiological resilience and nutrient utilization (El-Sayed, 2020) [13].

Feeding Rate and Conversion Efficiency

Feeding rate gradually declined over time in all groups, likely due to chronic ammonia exposure, which suppresses appetite and feeding behavior in fish. Despite this decline, *Spirulina*-supplemented groups—particularly those fed 1.2 g/100 L and 1.6 g/100 L—showed higher conversion efficiency than the control. Improved feed conversion efficiency suggests that *Spirulina* enhanced digestive enzyme activity and nutrient absorption, allowing fish to utilize feed more effectively even under stressful conditions. These findings are consistent with previous studies reporting improved feed efficiency in fish fed *Spirulina*-based diets (Promya & Chitmanat, 2011) [14].

Interestingly, the highest *Spirulina* level (1.6 g/100 L) did not consistently yield the best growth or feeding responses, indicating that excessive supplementation may not be beneficial. This supports the concept of an optimal inclusion level, beyond which no further physiological advantage is achieved.

Ammonia Concentration and Stress Mitigation

One of the most notable outcomes of this study was the significant reduction in ammonia concentration in tanks receiving higher Spirulina supplementation (1.2 g/100 L and 1.6 g/100 L). Spirulina may contribute to ammonia reduction through multiple mechanisms, including uptake of nitrogenous waste, enhancement of microbial activity, and improved fish metabolism that reduces ammonia excretion. Similar ammonia-reducing effects of microalgae in aquaculture systems have been reported by Azim *et al.* (2005)^[15] and Kumar *et al.* (2018)^[16]. Lower ammonia levels likely contributed to improved growth and feed efficiency, as ammonia is known to cause gill damage, oxidative stress, and impaired osmoregulation in fish (Randall and Tsui, 2002)^[2]. Therefore, Spirulina supplementation not only acted as a nutritional additive but also indirectly improved water quality, creating a more favorable rearing environment.

Colouration of Fish

Colouration is a critical quality trait in ornamental fish, and the present study revealed that Spirulina supplementation influenced pigmentation patterns in *I. sprengerae*. Fish fed 1.2 g/100 L Spirulina consistently showed improved muscle, fin, and skin colouration compared to lower supplementation levels. This effect is likely due to the high content of natural carotenoids, such as β -carotene and zeaxanthin, present in Spirulina, which are known to enhance pigmentation in fish tissues (Guroy *et al.*, 2012; Torrissen *et al.*, 1989)^[17, 18]. Although the control group showed higher fin and skin colour values in some months, this may be related to stress-induced pigment concentration rather than true enhancement of coloration quality. The consistently lower colouration observed in the 1.6 g/100 L group suggests that excessive Spirulina inclusion may interfere with pigment deposition or absorption, reinforcing the importance of optimal dosing.

Conclusion

Dietary supplementation of *Spirulina fusiformis* significantly improved growth performance, feed utilization, ammonia tolerance, and colouration in *Iodotropheus sprengerae* under ammonia-contaminated conditions. The 1.2 g/100 L inclusion level was optimal, producing the best overall growth, feed conversion, pigmentation, and water-quality improvement. Higher levels (1.6 g/100 L) reduced ammonia effectively but did not further enhance growth or colouration. These results indicate that Spirulina is a valuable functional feed additive for promoting health, performance, and aesthetic quality in ornamental fish culture.

References

1. Yuen Kip, Chew SF. Ammonia Production, Excretion, Toxicity, and Defense in Fish: A Review. *Frontiers in Physiology*,2010;1:134.
2. Randall DJ, Tsui TKN. Ammonia toxicity in fish. *Marine Pollution Bulletin*,2002;45(1):17–23.
3. Naeem Z, Zuberi A, Ali M, Naeem AD, Naeem M. Toxic effects of ammonia exposure on growth and hematological response of *Clarias batrachus* (Linnaeus, 1758). *Applied Ecology And Environmental Research*,2023;21(6):5055–5067.
4. Zou Y, Chen W, Xia B, Xiang Y, Shen Z, Han Y, *et al.* Ammonia toxicity in the bighead carp (*Aristichthys nobilis*): hematology, antioxidation, immunity, inflammation and stress. *Toxics*,2023;11(3):243.
5. Hardy R. Fish feed formulation. *Fish Feed Technology*,1980:233–239.
6. Abdelmageed M, Ali M, Mahmoud HA, Aldoma M, My H. Proximate composition of sudanese *Sesamum indicum* L. (white and brown) sesame seeds. *Archives of Organic and Inorganic Chemical Sciences*,2020;4:574–577.
7. Sprague JB. Measurement of pollutant toxicity to fish. III. Sublethal effects and safe concentrations. *Water Research*,1971;5:245–266.
8. Maynard AL, Loosli KJ. *Animal nutrition*, 1962.
9. Santhy KS. Rates of feeding and assimilation in the silkworm *Bombyx mori*, as influenced by methoprene. *Journal of Applied Pharmaceutical Science*,2015;5(10):101–106.
10. Wagade MS, Sharma SK, Sharma BK, Shivani, Keer NR. Effect of natural β -carotene from carrot (*Daucus carota*) and spinach (*Spinacia oleracea*) on colouration of swordtail (*Xiphophorus hellerii*). *Journal of Entomology and Zoology Studies*,2018;6(6):699–705.
11. Habib MAB, Parvin M, Huntington TC, Hasan MR. A review on culture, production and use of Spirulina as food for humans and feeds for domestic animals and fish. *FAO Fisheries and Aquaculture Circular*, 2008.
12. Abdel-Tawwab M, Ahmad MH. Effect of dietary protein regime during the growing period on growth performance, feed utilization, and whole-body chemical composition of Nile tilapia. *Aquaculture Research*,2009;40:1532–1537.
13. El-Sayed AFM. *Tilapia Culture*. 2020.
14. Promya J, Chitmanat C. The effects of Spirulina platensis and *Cladophora* algae on growth performance, meat quality and immunity of tilapia. *Songklanakarin Journal of Science and Technology*,2011;33:623–630.
15. Azim ME, Little DC, Bron JE. Microbial protein production in activated suspension tanks manipulating C:N ratio in feed and the implications for fish culture. *Aquaculture*,2005;252:125–137.
16. Kumar V, Sinha AK, Romano N, Allen KM, Bowman BA, Thompson KR, *et al.* Metabolism and nutritive role of carbohydrates in fish. *Reviews in Aquaculture*,2018;10:1–23.
17. Guroy B, Sahin I, Mantoglu S. Spirulina as a natural carotenoid source on growth, pigmentation, and reproductive performance of fish. *Aquaculture International*,2012;20:869–878.
18. Torrissen OJ, Hardy RW, Shearer KD. Pigmentation of salmonids—carotenoid deposition and metabolism. *Critical Reviews in Aquatic Sciences*,1989;1:209–225.