RESEARCH ARTICLE

Spirulina Arthrospira platensis as fishmeal replacement: effect on the growth, survival and proximate composition of Nile tilapia *Oreochromis niloticus*

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Abstract: With the aquaculture industry's growth comes an increased demand for fishmeal, leading to overfishing and rising prices. *Spirulina*, a microalga rich in protein and essential nutrients, is proposed as an alternative protein source research in the diet of Nile tilapia, *Oreochromis niloticus*, a major aquaculture species. This study investigates the effect of replacing fishmeal with *Spirulina* at a higher percentage than in previous studies. The formulated diets containing 0%, 30%, and 50% *Spirulina* were tested over a 58-day period to determine the effect on growth, survival, and proximate composition on Nile tilapia. Results showed that replacing fishmeal with *Spirulina* at both 30% and 50% levels did not negatively affect growth or survival rates. Water quality parameters remained within optimal ranges throughout the experiment, indicating that *Spirulina* inclusion did not adversely affect water quality. Proximate analysis of the fish muscle revealed no significant differences in crude protein content but fish fed 30% *Spirulina* had higher crude fat content. Overall, the study concludes that *Spirulina* can replace fishmeal up to 50% without negative effect on the growth, survival and proximate composition of Nile tilapia.
Keywords: *Spirulina*, fishmeal, tilapia growth, survival, proximate composition

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1 Introduction

The growth of the aquaculture industry in recent years for supplying animal food for humans has resulted in an increased demand for fishmeal and fish oil, which are used as feed ingredients due to their high protein content. This is especially true for carnivorous species such as grouper, Asian seabass or barramundi, and Atlantic salmon (Rosas et al., 2018; Geng et al., 2022). Even omnivore species such as tilapia are given formulated diets containing fishmeal. The demand for fishmeal and its cost have been rising over the years as aquaculture is increasing on a large scale (FAO, 2022). This has led to fisheries being overexploited, potentially causing overfishing, and consequently, the supply is insufficient. To address the substantial demand and reduce pressure on fishmeal, aqua-feed experts continue to search for other ingredients with high protein content, sustainable supply, and high nutritional value, such as algae, plants, and animal sources (Rosas et al., 2018; Geng et al., 2022;Woolley et al., 2023).

Spirulina *Arthrospira platensis* is one of the most commonly produced microalgae commercially. It is a filamentous and multicellular cyanobacterium that contains a high percentage of crude protein, approximately 65% by dry weight, and contains the essential amino acids (Koru E., 2012) and has minerals, antioxidants, vitamins, essential fatty acids, and various bioactive compounds (Zhang et al., 2020). Furthermore, these components help aquaculture species resist diseases and improve growth performance (Yousefi et al., 2022). In formulated diets for fish such as Nile tilapia, *Spirulina* effectively replaced animal-derived proteins and it maintained normal reproduction throughout three generations of the fish (Lu and Takeuchi,2004). *Spirulina* plays a significant role for Nile tilapia because it improves the immune response and fish flesh quality, acts as a growth promoter, increases the survival of fish, reduces the cost of fish feed, and improves the blood profile (Abdel-Tawwab M. and Ahmad M.H., 2019; Ibrahem et al., 2013; Noman S., 2018). Mahmoud et al. (2018) determined that 1% and 2% *Spirulina* supplement in the diet of Nile tilapia had no significant impact on growth, however, it improved Nile tilapia's resistance to the bacteria *Pseudomonas fluorescens*. Al-Mahrouqi et al. (2023) analyzed the effects of replacing fishmeal with 5%, 10%, and 15% *Spirulina* on the proximate composition and intestinal microbiota of Nile tilapia juveniles and reported a diversity of microbes in fish fed *Spirulina* with low abundance of pathogens and high abundance of beneficial microbes.

Studies have been conducted to determine the effect of fishmeal replacement with *Spirulina* on the growth of Nile tilapia *Oreochromis niloticus* and other species. Takeuchi et al. (2002) compared the effect of raw *Spirulina* and commercial diet on the growth and body composition of Nile tilapia and found that fish fed the commercial diet has a slightly higher growth rate, however, fish fed *Spirulina* had higher protein and polar lipid content, and lower ash content. Velasquez et al. (2015) tested the inclusion of *Spirulina* at 30%, 45%, 60%, and 75% and found that 30% is the optimal inclusion level that resulted in increased growth performance of Nile tilapia, whereas at 60% inclusion, there was no significant effect on fish growth and at 75%, the growth rate decreased. Youssef et al. (2023) conducted a study on the partial replacement of fishmeal by *Spirulina* (0%, 2.5%, 5%, and 10%) in the diets for Nile tilapia and showed that *Spirulina* enhanced the growth performance of Nile tilapia. However, in the case of another tilapia species *Oreochromis mossambicus* the inclusion of 20-100% *Spirulina* (*Arthrospira maxima*) did not improve growth performance (Olvera-Novoa et al.,1998). Similar results were obtained in the case of hybrid red tilapia (*O. niloticus x O. mossambicus*) with the inclusion of 5-20% *Spirulina Arthrospira maxima* (Ungsethapand et al.,2010). In contrast, 75% inclusion of *Arthrospira platensis* resulted in increased growth of the hybrid red tilapia. Similarly, *Arthrospira platensis* can replace up to 75% of the fishmeal in the diet of whiteleg shrimp *Litopenaeus vannamei* (Macias-Sancho et al., 2014). However, in the case of Nile tilapia, a maximum of 30% fishmeal replacement with *Spirulina Arthrospira platensis* has been reported to result in increased growth while 75% resulted in decreased growth. In our study, 50% fishmeal replacement with *Spirulina* was hypothesized as the optimum level of replacement in the formulated diet for Nile tilapia which is the third major aquaculture finfish species in the world (FAO, 2022). The study was conducted to determine the effect of *Spirulina* as a fishmeal replacement at 30% and 50% on fish growth, survival, and proximate composition of Nile tilapia fed the three diets.

2 Materials and Methods

2.1 Formulation and preparation of diets

The three diets were formulated to balance the crude protein, crude lipid, fiber, and ash for all diets at 36%, 6%, 3%, and 7%, respectively. Since the experimental fish were Nile tilapia juveniles, which require higher protein, 36% was used, similar to other research studies (Olvera-Novoa et al.,1998; Ibrahem et al., 2013; Velasquez et al.,2015) which used 35-36% crude protein in Nile tilapia formulated diets.

The dry feed ingredients were bought from Bio Products Company in Barka, Oman. The feed ingredients were weighed according to the feed formulation and mixed well together in a bucket. Fish oil was added and all ingredients were mixed well. Water was added gradually to the mixture until the mixture became coherent. The mixture was introduced into the meat mincer machine for pelletizing. The pellets were air-dried for two days, and the dried pellets were put in plastic jars and kept in the chiller. To preserve the quality, small quantities of feed were taken, weighed, recorded, and kept in small containers for daily feeding in each tank. Whenever these feeds were used up, another batch of feeds was taken, weighed, and recorded to calculate the number of feeds given to each tank.

2.2 Effect on growth and survival of Nile tilapia

The experiment was conducted over 58 days at the Department of Marine and Fisheries Sciences, College of Agricultural and Marine Sciences, at Sultan Qaboos University (SQU). The initial mean weight of the juvenile *Oreochromis niloticus* was (14.24 ± 4.28 g). Nine glass tanks, each containing 64.5 L of freshwater, were stocked with ten fish (=155 fish per m^3). The three formulated diets (0% *Spirulina*, 30% *Spirulina*, and 50% *Spirulina*) were tested in three replicates each and distributed randomly. The fish were fed at 2% body weight or until satiation two times per day (morning and afternoon). To maintain water quality, feces and any uneaten feeds were siphoned out three times per week. The weight and length of all fish in each tank were measured every month to monitor their growth and survival. Dissolved oxygen and temperature were measured daily using ProODO Optical Dissolved Oxygen measuring device, while pH was measured using a Teletemp Double Junction Waterproof pH Meters device. Ammonia was measured by Hanna Ammonia Medium Range Portable Photometer-HI96715, while nitrite and nitrate were measured using API Fresh Water Master test kit once a week.

At the end of the experiment, the number of surviving fish was counted, and the final weight and total length of each fish were measured using a digital balance and measuring board to determine the growth parameters for each diet. The following formulas were used:

- Weight Gain (WG) = Average Final Weight – Average Initial Weight
- Average Daily Gain (ADG) = (Average Final Weight – Average Initial Weight) / Days
- Specific Growth Rate (SGR) = [(*ln* Average Final Weight - *ln* Average Initial Weight)/days] x 100
- Length Increment = Average Final Total Length - Average Initial Total Length
- Feed efficiency (FE) = Weight Gain / Feed Intake
- Feed Conversion Ratio (FCR) = Feed Intake / Weight Gain
- Survival Rate (SR) = (Final fish number / Initial fish number) x 100%

Table 1. Feed formulation ingredients (%) for the three diets (T1: 0% *Spirulina*, T2: 30% *Spirulina*, and T3: 50% *Spirulina*

Ingredient (%)	T1 (0% Spirulina)	T2 (30% Spirulina)	T2 (50% Spirulina)
Fishmeal	34	23.8	17
Spirulina	0	10.2	17
Soybean Meal	26	26	26
Wheat Flour	8.5	7.5	3
Wheat Bran	18	21	22.5
Corn Starch	10	7	10
Fish Oil	3	4	4

Table 2. Growth and survival of Nile tilapia fed the three diets (T1: Control or 0% *Spirulina*, T2: 30% *Spirulina*, and T3: 50% *Spirulina*) during a 58-Day Culture Period. Values are means and standard deviation of three replicates.

Diet	T1 (0% spirulina)	T2 (30% spirulina)	T3 (50% spirulina)
Average Initial Weight (g)	14.2 ± 4.9	14.1 ± 4.1	14.5 ± 3.8
Average Final Weight (g)	34.9 ± 9.0	32.3 ± 11.4	32.5 ± 9.1
Average Weight Gain (g)	20.7 ± 4.1	18.2 ± 7.3	18 ± 5.3
Average Daily Weight Gain (g)	0.36 ± 0.1	0.31 ± 0.1	0.31 ± 0.1
Average Specific Growth Rate (%)	1.6 ± 1.1	1.4 ± 1.7	1.4 ± 1.5
Average Initial Total Length (cm)	9.2 ± 0.9	9.0 ± 0.9	9.1 ± 1.0
Average Final Total Length (cm)	12.9 ± 1.1	12.1 ± 1.4	12.3 ± 1.3
Average Length Increment (cm)	3.7±0.2	3.1 ± 0.5	3.2 ± 0.3
Average Feed Intake (g)	29.2 ± 4.6	28.7 ± 2.2	28.8 ± 4.9
Average Feed Efficiency	0.7 ± 0.9	0.6 ± 3.4	0.6 ± 1.1
Average Feed Conversion Ratio	1.4±1.1	1.6 ± 0.3	1.6 ± 0.9
Average Survival Rate (%)	100 ± 0.0	100 ± 0.0	100 ± 0.0

2.3 Proximate analysis of fish muscle

At the end of the experiment, three fish were taken from each treatment, which all had the same size. They were put in plastic bags, labeled, and placed in the chiller at a temperature of -18°C until further analysis. For analysis, fish fillets were cut to extract only the flesh and then weighed. Then they were placed on a labeled plate then put in the oven at 60°C for 24 hours to get dried flesh and weighed again to calculate the moisture. Moreover, the dry muscles were ground in a blender to obtain a powder content for analysis. Proximate analysis for crude protein, crude lipid, and ash was performed using AOAC on triplicate samples in the feed laboratory.

2.4 Statistical Analysis

Analysis of Variance (ANOVA) and Tukey tests were performed to determine any significant difference ($P < 0.05$) in the water stability, attractiveness, palatability, and growth parameters.

3 Results and Discussion

3.1 Growth and survival rates

Table 2 shows the means and standard deviations of the growth of fish given the three treatments (T1, T2, and T3). The ANOVA and Tukey tests have shown no significant differences among the treatments ($P > 0.05$). The survival rate was 100% in all treatments.

Our results showed no significant difference in growth and survival of fish fed the three formulated diets using fishmeal and *Spirulina* as protein sources. In the study by Olvera-Novoa et al. (1998), who tested the replacement of fishmeal with *Spirulina* at ratios of 20, 40, 60, and 100%, there was no significant difference in growth at 20 and 40% replacement, and lower growth at 60, 80, and 100%. Velasquez et al. (2015) recommended replacing 30-45% of fishmeal with *Spirulina* in Nile Tilapia diets, and our study shows good results even at 50% fishmeal replacement. *Spirulina* is also reported to enhance growth in other species such as abalone Haliotis midae (Britz, P., 1996), dwarf gourami *Trichogaster lalius* (Baksi et al., 2017), and sturgeon Acipenser baeri (Palmegiano et al., 2005).

In future experiments, it may be worthwhile to test replacements higher than 50% of fishmeal and investigate other effects of using *Spirulina* as fishmeal replacement in the diet of other fish species (such as koi carp) that are more expensive species than tilapia.

3.2 Water Quality

Table 3 shows the minimum and maximum values in water quality parameters. There was no significant difference ($P > 0.05$) in the water quality parameters between treatments. Temperature increased during the experiment, which was

Table 3. Minimum and maximum water quality parameters in the rearing tanks for the evaluation of the three diets (T1: control; T2: 30% spirulina; T3: 50% spirulina) for 2 months.

Diet	Temperature (℃)	Dissolved Oxygen (mg/L)	pH	NH_3 (ppm)	NO_2 (ppm)	NO_3 (ppm)
T1 (0% spirulina)	20.7 ± 0.3 - 32.8 ± 0.4	1.9 ± 0.5 -8.03 ± 0.2	6.1±0.2 - 9.4±0.5	0.0 ± 0.0 -12.2 ± 0.0	0.0 ± 0.0 -30.0 ± 20.0	0.0 ± 0.0 -250.0 ± 0.0
T2 (30% spirulina)	20.6 ± 0.1 - 32.3 ± 0.5	1.8 ± 0.0 -8.4 ± 0.0	6.2±0.2 - 9.2±0.4	0.0 ± 0.0 -12.1 ± 0.0	0.0 ± 0.0 - 10.0 ± 0.0	0.0 ± 0.0 - 250.0 ± 0.0
T3 (50% spirulina)	20.7 ± 0.3 - 32.4 ± 0.1	1.8 ± 0.4 - 8.1 ± 0.22	6.3 ±0.5 - 9.2±0.4	0.0 ± 0.0 -11.8 ± 0.7	0.0 ± 0.0 -17.5 ± 28.2	0.0 ± 0.0 -250.0 ± 0.0

conducted towards summer. The dissolved oxygen fluctuated during the experiment period, and overall, there was a declining trend. The pH was in the range of 6.2-9.4, and there was a decreasing trend during the experiment period. There was no significant difference ($P > 0.05$) in ammonia, nitrite, and nitrate among the treatments. The water quality parameters during the experiment were similar among treatments and were generally within the optimal range for Nile tilapia.

3.3 Proximate composition of Nile tilapia muscles

Table 4 shows the crude protein, fat, ash, and nitrogen-free extract of the fish fed the control diet (T1), 30% *Spirulina* (T2), and 50% *Spirulina* (T3). There was no significant difference ($P > 0.05$) among the treatment means except in crude fat and NFE as indicated by ANOVA and Tukey tests. T1 had the highest NFE (10.67 ± 0.07%) whereas 30% *Spirulina* (T2) had the highest crude fat (5.58 ± 0.08%) and lowest NFE (9.63 ± 0.12%). On the other hand, the 50% *Spirulina* (T3) diet had the lowest crude lipid (4.6 ± 0.2%).

Table 4. Proximate composition of Nile tilapia Fed Three Diets (T1: Control or 0% *Spirulina*, T2: 30% *Spirulina*, and T3: 50% *Spirulina*). Values in a column with different superscripts are significantly different at $P<0.05$).

Diet	Crude Protein	Crude Fat (%)	Ash (%)	NFE (%)
T1	66.70 ± 0.41	4.92 ± 0.13^{b}	12.47±0.01	10.67±0.07^{a}
T2	59.59 ± 0.73	5.58 ± 0.08^{a}	10.78±0.16	9.53±0.12^{c}
T3	62.88 ± 1.38	4.56 ± 0.19^{b}	12.52±0.73	10.06±0.22^{b}

Our results showed no significant difference in crude protein in the muscle of fish, reflecting the isonitrogenous content of the diets, and are similar to the findings of Al-Mahrouqi et al. (2023) and Ungsethaphand et al. (2010). The higher lipid in fish fed T2 (30% *Spirulina*) could be due to the combination of the lipids in fishmeal and *Spirulina*, while the higher ash in fish fed T1 (fishmeal) may be due to the fish species used or possibly the inclusion of fish bones in preparing the fishmeal. Lu et al. (2004) reported that tilapia fed *Spirulina* contain high protein, polar lipids, and omega-6 HUFA, and lower amounts of non-polar lipid, all of which demonstrate healthy flesh quality.

4 Conclusions

Fish meal replacement with *Spirulina* at 30 and 50% in the formulated diet for Nile tilapia is appropriate because there was no negative effect on the growth, survival, and proximate composition. The use of *Spirulina* to partially replace fishmeal could decrease the pressure on the wild fisheries for fishmeal production. Future research could explore higher replacement levels and the effects of *Spirulina* inclusion in the formulated diets for other aquaculture species.

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Author Contributions

R.A.N.: Data curation and analysis, Visualization, Writing-Original draft preparation.

W.G.G.: Conceptualization, Methodology, Validation, Supervision, Project administration, Writing-Reviewing and Editing.

A.A.S.: Conceptualization, Methodology, Validation, Writing-Reviewing and Editing.

Conflict of Interest

The authors declare no conflict of interest.

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