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Spirulina (*Arthrospira fusiformis*, Gomont 1892) as a High-Quality Feed Ingredient for Nile Tilapia Fingerlings

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Abstract

A partially randomized experimental design was conducted to evaluate the effect and potential of spirulina species (*Arthrospira fusiformis*) isolated from Momela Lakes, Tanzania, on the growth, antioxidant capacity, feed utilization, and body composition of Nile tilapia fingerlings. Five dietary treatments were formulated, including spirulina meal at 5%, 10%, and 15% of the total diets, as well as control diets with freshwater shrimp or Lake Victoria sardine. The protein content of the experimental diets was 25, 28, and 35% for a treatment containing spirulina at 5, 10, and 15% inclusion, respectively, while the control diets had 35% crude protein each. After eight weeks of feeding, fish fed with a lower inclusion level of spirulina showed the best growth performance and feed utilization efficiency, similar to the fish fed with control diets. The group fed with 5% spirulina exhibited excellent protein efficiency ratio compared to all other fish groups. All spirulina-fed fish groups demonstrated higher protein deposition and strong antioxidant activity against free radicals compared to those fed with control diets. In conclusion, spirulina proves to be a superior feed ingredient for high-quality Nile tilapia farming, providing comparable results to control feeds.

Keywords: Spirulina, *Arthrospira fusiformis*, Nile tilapia fingerlings, Feed utilization efficiency, Antioxidant capacity.

Introduction

Tilapia is a popular fish for aquaculture worldwide due to its adaptive feeding behaviour and ability to consume a wide range of feeds. As the fish matures, its feeding preference shifts towards plant materials (Takeuchi et al. 2002). Fishmeal has traditionally been used in fish diets as a source of protein (Kathleen 2016), however, due to increasing demands for fish and rising prices (Kim et al. 2013, Nguyen and Kinnucan 2018, Garba 2021), there is

currently a need for alternative, affordable protein sources. In Tanzania, like many Sub-Saharan countries, the aquaculture sector faces various challenges, including barriers related to fish feeds (Munguti et al. 2014, Tran et al. 2019, Mmanda et al. 2020, Mulokozi et al. 2020, Mramba and Kahindi 2023). Prohibitive costs of commercial feeds and fish-based ingredients lead to unprofitable practices for the majority of fish farmers who rely on natural productivity with minimal supplementation (Kaliba et al. 2006,

Chenyambuga et al. 2014). Introducing a cheap alternative protein source is crucial for sustainable aquaculture production in Tanzania and other economically-similar countries.

Studies have shown that plant-based diets can significantly replace fishmeal and reduce feed ingredient costs (Madalla 2008, El-Sheekh et al. 2014, Khalila et al. 2018). Microalgae, particularly *Arthrospira* species (commonly known as spirulina), have been found to have multiple benefits for farmed fish. They can replace fishmeal, improve fish health, enhance meat quality, and provide essential nutrients (El-Sheekh et al. 2014, Pilot et al. 2014). Spirulina is considered as a nutrient-rich microalgae with high quality protein having all essential amino acids, fats and polyunsaturated fatty acids, vitamins and minerals, as well as the phytopigments that protect the body against free radicals (Takeuchi et al. 2002, Hosseini et al. 2013, Michael et al. 2019). Previous studies have utilized different species of spirulina in aquaculture as feed additives and for partial fishmeal replacement (James et al. 2006, Belal and El-Hais 2012, Hussein et al. 2013, Mulokozi et al. 2019a). In Tanzania, *Arthrospira fusiformis* is abundant in soda lakes located in the northern part of the country, serving as a food source for birds like the Lesser Flamingo (Hamisi et al. 2017, Mulokozi et al. 2019b). This study therefore focused on using *A. fusiformis* isolated from Momela Lakes for Nile tilapia fingerlings farming, by evaluating its effects on growth performance, body composition, and feed utilization efficiency.

Materials and Methods

Source of microalgae and cultivation

The inoculum of *Arthrospira fusiformis* was obtained from the stock culture kept at the Institute of Marine Sciences, University of Dar es Salaam, Tanzania, which was previously isolated from the Lake Big Momela (Mulokozi et al. 2019b). At the Institute, the culture was maintained in Zarrouk medium in Erlenmeyer flasks. Before the onset of this study, the culture was checked for contamination and if noticed, a

serial dilution process was conducted to ensure unialgal culture. The mass culture of spirulina was conducted in plastic basins (100 litres) using a low-cost medium produced by mixing NPK complex fertilizer and few ingredients of Zarrouk's media (Michael et al. 2019). The culture was agitated twice a day by using a clean wooden spoon to avoid clumps. After one month, the biomass was harvested, rinsed with clean fresh water to remove adsorbed salts then sundried, followed by grinding to powder. The dry-ground biomass was then used in the feed formulation of the fish diets.

Proximate composition analysis

The proximate analysis for the determination of nutritive compositions in the feed ingredients, experimental diets (Table 1), and fish's whole body (Table 4) was done at the Department of Botany, University of Dar es Salaam based on the standard analytical methods (Allen 1989, AOAC 1999, Michael et al. 2019). Whole body proximate composition of the experimental fish was determined at the end of the experiment, except for the initial body composition of fish taken from each tank before the experiment. The moisture content was determined by oven drying the samples at 60 °C overnight to a constant weight. The percentage loss in weight after drying was the moisture of the sample while the remaining was the dry matter. Protein content was analysed by semi-micro Kjeldahl digestion followed by the indophenol-blue colorimetric method. The crude lipid was analysed using a mixture of organic solvents containing chloroform, methanol, and water in the ratio of 1:2:0.8, which was then raised to 2:2:1.8 (Bligh and Dyer 1959). The indigestible materials, termed as fibres were determined gravimetrically after acid hydrolysis and alkali extraction method. The other content of the sample was ash, which is a measure of the total minerals. This was determined as the residue after combustion of the dried sample materials.

Experimental diets

Five dietary treatments were formulated using ingredients shown in Table 2: three with spirulina and two control diets with fishmeal. The spirulina diets had 5% (T5), 10% (T10), and 15% (T15) per 100 g diet, while the control diets consisted of either freshwater shrimp meal (TC), accounting for 51% of the total diet, or Lake Victoria sardine (TR), representing 65% of the total diet.

These ingredients were selected because they are among the commonly used protein sources in some locally-formulated diets. Table 1 presents the proximate composition of the feed treatments, with the 5% spirulina diet (T5) containing 25% crude protein, the 10% spirulina diet (T10) containing 28% crude protein, and the 15% spirulina diet (T15) having a similar protein content (35%) to the control diets.

Table 1: Proximate composition of the experimental diets

Composition (%)	Diets				
	T5	T10	TC	TR	T15
Crude protein	25.1	28.3	35.1	35.4	35.2
Moisture	5.98	6.88	7.92	7.28	5.79
Crude lipid	9.06	11.62	6.55	11.70	13.99
Crude fibre	7.93	11.74	6.28	4.87	13.70
Ash	10.27	10.82	18.61	12.02	10.91
Nitrogen free extract	38.70	31.56	26.88	33.53	16.46
Dry matter	94.0	93.12	96.31	97.54	94.21

Table 2: Feed ingredients used in the formulation of experimental diets

Ingredient (g 100 g ⁻¹)	Experimental diets				
	T5	T10	TC	TR	T15
Maize bran	85	78	41	27.0	70
Binder (Pollard)	3.0	3.0	3.0	3.0	3.0
Sunflower seed cake	5.0	7.0	3.0	3.0	10.0
Premixes*	2.0	2.0	2.0	2.0	2.0
Spirulina meal	5.0	10.0	-	-	15.0
Fishmeal (sardine)	-	-	-	65.0	-
Fishmeal (shrimp)	-	-	51.0	-	-

*Premixes of vitamins, minerals and amino acids (T5 = Dietary treatment with 5% spirulina, T10 = Dietary treatment with 10% spirulina, TC = control diet with shrimp meal, TR = control diet with sardine meal, T15 = Dietary treatment with 15% spirulina).

Stocking and experiment setup

The experiment was conducted for eight weeks in the concrete tanks of 1 m³ located at Kunduchi Fisheries Centre, University of Dar es Salaam. The Nile tilapia fingerlings, *Oreochromis niloticus* were obtained from a stock kept in concrete ponds whose brood stocks were collected from Tanzania Fisheries Research Institute (TAFIRI) Mwanza. In stocking, 165 healthy mixed sex fingerlings of 4.1 ± 0.1 g average weight were selected, and randomly distributed into five dietary groups. Each treatment had three replicates with a stock of 11 fingerlings per tank. Prior to the initial feeding, the

fingerlings were subjected to a two-day period of fasting to ensure their guts were empty. Subsequently, one fish from each tank was selected and frozen for the initial proximate composition analysis. The remaining 10 fingerlings in each tank were fed three times a day (at 9 am, 12 pm and 4 pm), except for the day before and after the sampling for weight measurement when the fingerlings were fed only once to minimize any potential handling stress. At first, the fingerlings were fed at 10% of their wet body weight followed by 7% after two weeks and finally 5% up to the end of the experiment. Water exchange was done at 50–75% by

volume every three days. Water quality variables such as dissolved oxygen (Ecosense DO 200A, China), temperature and pH (Combo HI98129, Italy) were measured *in-situ* daily.

Growth performance and feed utilization indices

The body weight was group-weighed on a weekly basis for each tank by using a portable balance (Endel, Global Weighing Company) and feed amount adjusted accordingly. Siphoning was done daily early in the morning before feeding to remove the faeces and other wastes in the tank.

AWG (g) = $FW - IW$	1
DWG (g fish day ⁻¹) = $(FW - IW)/Time (days)$	2
SGR (% day ⁻¹) = $100 \times (\ln[FW] - \ln[IW]) / (Time (days))$	3
Survival (%) = $100 \times (Available\ stock / Fish\ stocked)$	4
FI (g fish day ⁻¹) = $(Total\ feed\ intake\ per\ fish / Time(days))$	5
FCR = $Feed\ intake / live\ weight\ gain$	6
FE percent (FE %) = $100 \times (Weight\ gain / Feed\ intake)$	7
PER = $Live\ weight\ gain / Protein\ intake$	8

Where; FW = Final fish weight, IW= Initial fish weight.

Body composition and antioxidant capacity of the experimental fish

At the end of the experiment, nine (9) fish from each dietary treatment were randomly selected for the analysis of the whole-body proximate composition. All fish samples were first weighed and then oven dried to constant weight, thereafter ground for the analysis of crude protein, crude lipid, ash and nitrogen free extracts, following standard procedures (Allen 1989, AOAC 1999). On the other hand, the antioxidant activity was determined in terms of radical scavenging activity (RSA) and effective concentration at which 50% (EC50) of the free radicals are scavenged. Three fish from each dietary treatment were dissected to remove the liver and gut for testing the scavenging ability against the free radical, 2,2-diphenylpicrylhydrazyl (DPPH). The entails were smashed and placed in the solution containing 95% ethanol, for digestion. The extracts for each fish group were then diluted into four different concentrations ranging from 0.001 to 0.03 mg/ml from which, 3 ml of the concentration was mixed with 1 ml of

Unconsumed feeds were collected from the feeding point by siphoning after one hour of feeding and left to sun dry and then weighed for estimating the actual feed intake. Growth performance was measured in terms of average weight gain (AWG), average daily weight gain (DWG), specific growth rate (SGR) and survival. The utilization of feed was measured as the feed intake (FI), feed conversion ratio (FCR), feed efficiency percent (FE%) and protein efficiency ratio (PER). The protein intake was estimated by calculating the protein content in the fish feed. The following formulas were used:

DPPH (0.125 μM in 95% ethanol). The inhibitory capacity of the gut extract against DPPH was calculated according to equation 9;

$$\%RSA = ((A_{DPPH} - AE) / A_{DPPH}) * 100 \dots 9$$

Statistical analyses

All statistical analyses were carried out using the SPSS Statistical software version 20 (IBM SPSS Statistics 20, IBM Corp. 2011, USA). Normality was tested with the Kolmogorov–Smirnov test, and homogeneity of variance was assessed with Levene’s test. One-way ANOVA determined differences in treatment means for proximate composition, growth, and feed utilization parameters. Repeated measure ANOVA examined differences in fish daily weight. The Tukey Honest Significant Difference test (THSD) was used for multiple mean comparisons when significant differences existed between feed treatments. Significance was determined at $p \leq 0.05$. Results are reported as mean ± standard deviation.

Results

Fish growth performance

All experimental fish showed an increase in body weight with time (Figure 1). The fish group fed with low amount of spirulina inclusion (T5) showed the best growth similar to the control fish groups (Table 3). The fish in treatment T5 had their SGR statistically similar to both of the control diets, and similar FW, AWG and DWG with feed containing sardine (TR). There was a poor growth trend for the fish group fed with higher amounts of spirulina thereby recording lower values of all the measured growth parameters. The growth was intermediate for the fish group fed on 10% spirulina inclusion, while TC showed the best growth. There was a statistically significant influence of feed treatments ($F(4, 10) = 84.203, p < 0.001$), experimental time ($F(4, 10) = 53.526, p = p < 0.001$), and a synergetic influence of experimental period and feeding treatment ($F(3, 39) = 3.223, p = p < 0.001$) to the DWG of the experimental fish. On the other hand, the survival rate was above 80% for all

experimental fish with spirulina fed fish groups recording the excellent survival of up to 96% compared to the control which had 85% as the highest record. There was no significant variation among the feed treatments ($F(4, 10) = 1.204, p = 0.380$), and there was no mortality related with the experimental diet.

Feed utilization parameters

There was a significant difference in the feed utilization efficiency among the fish groups (Table 3). Similar to growth performance, fingerlings fed lower inclusion levels of spirulina exhibited good feed intake. However, those fed higher inclusion levels showed reluctance to consume the feeds, resulting in poor FCR and PER. Notably, the fish group T5 exhibited FCR that was statistically comparable to the control diets. Remarkably, the fish group that consumed the diet containing 5% spirulina achieved the highest PER, followed by the 10% spirulina-fed fish groups, which displayed comparable efficiency to the control fish groups.

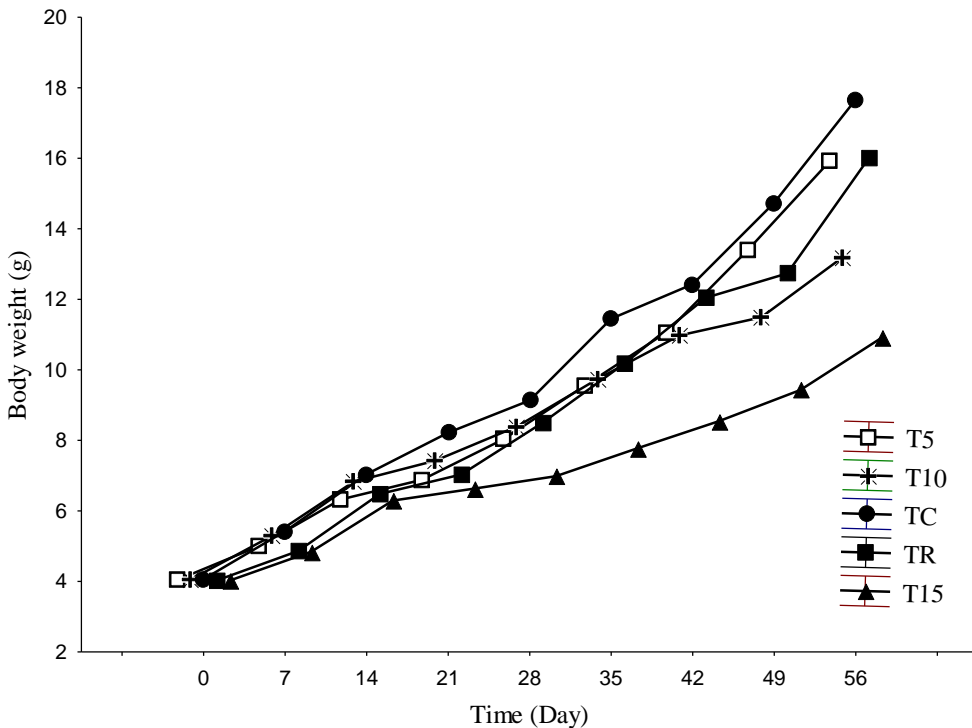


Figure 1: Changes in fish body weight during the eight weeks of the experiment.

Table 3: Fish growth and feed utilization parameters (Mean ± SD, n = 3) in the experimental set-up

Parameter	Dietary treatments				
	T5	T10	TC	TR	T15
IW (g)	4.1 ± 0.2 ^a	4.1 ± 0.1 ^a	4.1 ± 0.1 ^a	4.0 ± 0.1 ^a	4.0 ± 0.1 ^a
FW (g)	14.6 ± 0.5 ^a	12.3 ± 0.4 ^b	16.4 ± 0.3 ^c	15.6 ± 0.3 ^{ac}	10.5 ± 0.2 ^d
AWG (g)	10.6 ± 0.8 ^a	8.2 ± 0.7 ^b	12.3 ± 0.4 ^c	11.6 ± 0.6 ^{ac}	6.5 ± 0.4 ^d
DWG (g fish day ⁻¹)	0.19 ± 0.01 ^a	0.15 ± 0.01 ^b	0.22 ± 0.01 ^c	0.21 ± 0.01 ^{ac}	0.12 ± 0.01 ^d
SGR (% day ⁻¹)	2.3 ± 0.10 ^a	1.9 ± 0.1 ^b	2.5 ± 0.1 ^a	2.4 ± 0.1 ^a	1.7 ± 0.1 ^c
Survival (%)	89.3 ± 11.1 ^a	88.4 ± 0.8 ^a	82.9 ± 5.1 ^a	85.5 ± 4.8 ^a	96.3 ± 6.4 ^a
FI (g fish day ⁻¹)	0.4 ± 0.0 ^a	0.4 ± 0.0 ^b	0.5 ± 0.0 ^c	0.4 ± 0.0 ^d	0.4 ± 0.0 ^e
FCR	2.1 ± 0.2 ^a	2.6 ± 0.24 ^b	2.1 ± 0.1 ^a	2.0 ± 0.2 ^a	3.1 ± 0.2 ^c
PER	1.9 ± 0.1 ^a	1.4 ± 0.1 ^b	1.4 ± 0.0 ^b	1.4 ± 0.1 ^b	0.9 ± 0.1 ^c

Different superscripts in the same row indicate the significant difference in the means ($p < 0.05$). Where: IW stands for initial weight, FW for final weight, AWG for average weight gain, DWG for daily weight gain, SGR for specific growth rate, FI for feed intake, FCR for feed conversion ratio, PER for protein efficiency ratio.

Fish body composition

There was a significant difference in the whole-body proximate composition for the crude protein ($F(4, 10) = 40.219, p < 0.001$), ash ($F(4, 10) = 169.26, p < 0.001$) and crude lipid ($F(4, 10) = 2.969, p = 0.039$). The spirulina-fed fish groups deposited higher protein than the control groups, and the fish that were offered low levels of spirulina

inclusion had higher protein content (Table 4). Surprisingly, the fish fed with the sardine meal deposited the least crude protein than that of the initial fish. Similarly, for the ash content, the amount of the final body was lower compared to the initial content. The body of the fish fed with shrimp meal recorded the lowest values of ash content.

Table 4: Whole-body chemical composition (on dry weight basis) of the Nile tilapia fingerlings before and after the feeding experiment (Mean ± SD, n = 3)

Compositi on (%)	Initial	Dietary treatments				
		T5	T10	TC	TR	T15
MO	73.1 ± 2.2	73.9 ± 3.4 ^a	75.2 ± 3.8 ^a	73.2 ± 0.3 ^a	75.4 ± 1.6 ^a	75.0 ± 2.0 ^a
DM	26.9 ± 2.2	26.1 ± 3.4 ^a	24.8 ± 3.8 ^a	26.8 ± 0.3 ^a	24.7 ± 1.6 ^a	24.9 ± 2.0 ^a
CP	49.3 ± 0.4	51.4 ± 1.2 ^a	51.9 ± 0.7 ^a	49.0 ± 0.4 ^a	41.4 ± 2.1 ^b	50.4 ± 0.6 ^a
CL	14.6 ± 1.8	11.1 ± 1.8 ^{ab}	11.7 ± 2.3 ^a	12.6 ± 2.9 ^a	11.2 ± 2.0 ^a	15.1 ± 2.7 ^{ac}
Ash	27.8 ± 2.5	24.4 ± 0.5 ^a	24.1 ± 0.6 ^a	15.7 ± 0.5 ^b	23.3 ± 0.6 ^{ac}	25.2 ± 0.6 ^{ad}

Different superscripts in the same row indicate the significant difference in the means ($p < 0.05$). Where: MO = moisture, DM = dry matter, CP = crude protein, CL = crude lipid.

Antioxidant activity of the fish

All the fish groups fed on spirulina had stronger scavenging ability than the control fish, and strongest capacity was expressed by T10, and similarly the lowest effective concentration (EC_{50}) at which 50% of the DPPH free radicals were quenched (Figure 2). Poor scavenging ability was recorded in

TR, which also attained the EC_{50} at the highest concentration of 0.012 mg/ml while others attained EC_{50} at lower concentrations (Figure 2). The radical scavenging ability varied significantly with extract concentration ($F(1, 4) = 99.667, p = 0.000$), and fish groups ($F(1, 4) = 8.259, p = 0.000$).

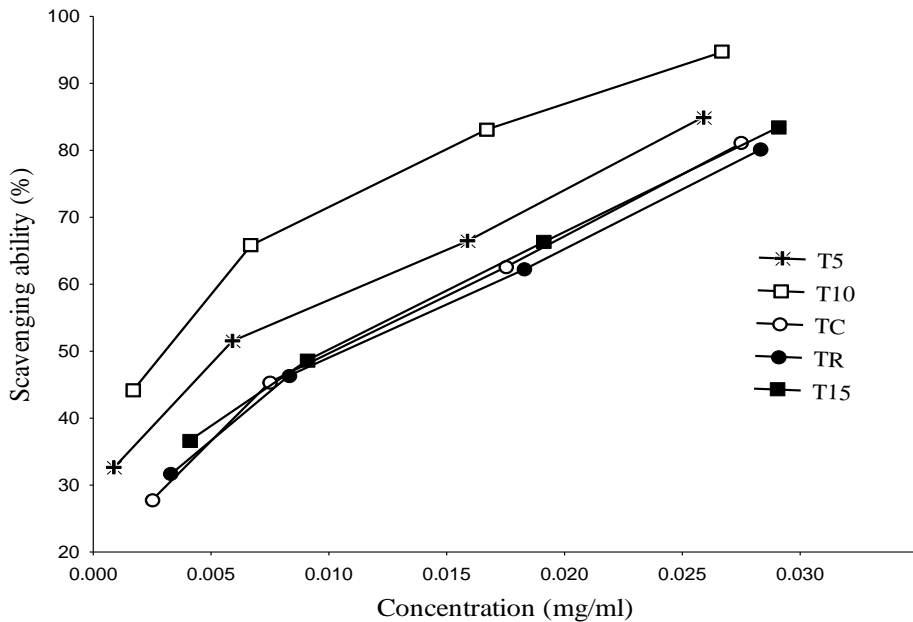


Figure 2: The scavenging ability of experimental fish's extracts against DPPH.

Discussion

In the present study, the amounts of protein in spirulina-containing diets ranged between 25.1 and 35.2%, and the reference diets contained 35% of the crude protein. This was in agreement to what was earlier suggested by Bahnasawy (2009) that the Nile tilapia fingerlings (5 to 25 g) had the protein requirement ranging from 25 to 35%. The study revealed that with spirulina as one of the feed ingredients, the amount of protein in the feed does not determine the biochemical composition of the fish, as the protein content of the fish's whole-body did not vary among the feed treatments. This can be linked to the changes in the rate of protein synthesis and deposition in the fish's body and muscles (Carter et al. 2011). The spirulina-fed fish groups exhibited higher protein deposition compared to the control groups, which had a large quantity of protein. Also, the fish that were offered low levels of spirulina inclusion had higher protein content than that fed on high content. Similar findings have been reported in previous studies. For instance, Bin Dohaish et al. (2018) found that Nile tilapia supplemented with lower levels (5–20%) of *Spirulina platensis* had higher

protein content in their bodies compared to the control and fish fed with 50% spirulina. A similar scenario was also observed by Mulokozi et al. (2019a) where the highest protein composition (53.48%) was recorded in the body of Rufiji tilapia supplemented with 25% spirulina while the fish fed with 100% spirulina had 51.18% protein.

The composition of lipids increased with the level of spirulina inclusion in the diet whereas the fish which consumed high inclusion, deposited higher levels of lipids. This tendency is similar to what was observed by Tongsir et al. (2010) who found that the Mekong Giant Catfish fed with higher spirulina inclusion had higher composition of protein and lipid. However, the results are contrary to those recorded by Abdulrahman and Ameen (2014) who found that lipid content in the body of common carp was reduced as spirulina was increased in the diet. The values of moisture content were in the acceptable level (60–80%), which concur with many of the previous studies (El-Sheekh et al. 2014, van Ruth et al. 2014, Teame et al. 2016). However, fish groups fed on a lower amount of spirulina (5%) and those fed on shrimp meal had a good quality meat than

other groups, as they contained lower moisture contents.

The lower feed intake in spirulina diets containing higher inclusion levels was possibly attributed to high fibre content (Table 1) and palatability as also previously reported by Abdel-Tawwab et al. (2006). Spirulina meal has no attractant smell, unlike fishmeal and shrimp meal, therefore an increase in spirulina inclusion reduces the palatability, which in turn affects the feed intake as was observed in this study. The FCR of 5% spirulina-fed fish was statistically similar to the control diets signifying the capacity of spirulina to improve feed efficiency by triggering the intestinal bacteria (Amer 2016). Previous studies have revealed the capacity of spirulina addition in the diet of Nile tilapia showing great impact in the FCR even at a low level of inclusion. For instance; Belal and El-Hais (2012) found that the addition of 1% spirulina (*A. fusiformis*) to the fishmeal-based diet, led to an excellent FCR of 1.27. Similarly, the study conducted by Bin Dohaish et al. (2018) recorded a FCR of 0.78 with an inclusion of a 5% spirulina. It was found that the fish fed with higher spirulina inclusion had lower PER. This suggests that the fingerlings were able to take the feed but failed to assimilate it completely (Takeuchi et al. 2002). Furthermore, Bahnasawy (2009) reported a more likely scenario whereby feed with low protein content was assimilated efficiently and resulted in good PER, suggesting that fish do take an amount of protein which they can assimilate efficiently. The tendency of PER observed in the current study however, is similar to that reported by Kim et al. (2013) for the Parrot fish (*Oplegnathus fasciatus*) and Mulokozi et al. (2019b) for the Rufiji tilapia fry (*Oreochromis urolepis urolepis*) that also recorded higher protein efficiency ratios at lower spirulina inclusion. On the contrary however, our findings differ from those reported by James et al. (2006) who found that the PER increased with the level of spirulina in the diet of the Red Swordtail. Other studies have reported an inconsistent tendency; for instance, El-Sheekh et al. (2014) observed that the hybrid red tilapia fed

75% spirulina had similar PER to that fed 100% spirulina, while Amer (2016) showed that the supplementation at 1% spirulina had better PER than 0.5 and 1.5% inclusions.

Spirulina is among the antioxidant-rich microalgae due to possession of phytopigments and vitamins (Takeuchi et al. 2002, Michael et al. 2018). Antioxidants usually exhibit powerful scavenging capacity towards the free radicals, as it was revealed that spirulina-fed fish possessed higher scavenging ability compared to the control fish groups. These results agree with those from previous studies which show the antioxidant potential of spirulina to farmed fish (James et al. 2006, Kim et al. 2013, Amer 2016). However, it was noticed that the scavenging activity was not related to the amount of spirulina content as the fish fed with higher inclusion had lower ability compared to those fed with lower inclusion, the reason for this observation remains unclear.

Conclusion

The study found that fish groups fed on low levels of spirulina-containing diets expressed the best growth, and feed utilization efficiency, similar to the control diets. It was further noticed that high amount of spirulina inclusion did not result in improved growth or feed efficiency, rather, a small amount was sufficient for significant improvements. The study therefore recommended spirulina, *Arthrospira fusiformis* as an ideal feed ingredient for raising better-quality Nile tilapia fingerlings.

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Declaration of Conflict of Interest

Authors declare that there is no conflict of interest.

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