

Full Length Research Paper

# Use of spirulina (*Arthrospira fusiformis*) for promoting growth of Nile Tilapia fingerlings

Elsayed B. Belal<sup>1\*</sup>, Khalafalla, M. M. E.<sup>2</sup> and El-Hais A. M. A.<sup>3</sup>

<sup>1</sup>Department of Agricultural Microbiology, Agricultural Botany, Faculty of Agriculture, Kafrelsheikh University, 33516, Kafr El-Sheikh, Egypt.

<sup>2</sup>Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, 33516 – Kafr El-sheikh, Egypt.

<sup>3</sup>Department of Animal Production, Faculty of Agriculture, Tanta University, Egypt.

Accepted 30 July, 2012

In the present investigation, spirulina (*Arthrospira fusiformis*) was used as feed additives on growth performance in Nile Tilapia (*Oreochromis niloticus*). *A. fusiformis* was isolated from various rook pool of a rocky shore, Alexandria and Kafr El-Sheikh Governorates, Egypt. The results showed that the optimum medium, pH and temperature for the growth protein and chlorophyll-a content of *A. fusiformis* were Zarrouk medium, 9 and 35°C, respectively. The dry weight of *A. fusiformis* was 1.8 g/L and protein and chlorophyll-a contents were 64.3% and 13.2 mg/g at 35°C and pH 9, respectively. *A. fusiformis* was used as feed additives on growth performance in Nile Tilapia for 12 weeks. Three (3) diets were formulated to be isocaloric and isonitrogenous containing (about 4616 kcal/kg DM) and (about 31.49% CP) for Tilapia fingerlings: one, a control diet without supplements; second, a supplement with 1% *A. fusiformis* and third, a supplement with 1% *Spirulina pacifica*. Results indicated that fish fed on 1% spirulina exhibited greater growth than those fed with the control diet. Fish fed with the control diet had the lowest protein content. Carcass lipid recorded the highest value in the control treatment, which was statistically different from the supplemented treatments. Ash content increased significantly with the increase spirulina levels as compared with the control treatments. Fish fed with spirulina-containing exhibited higher glucose, lipid and protein values in fish serum. Also, fish fed with spirulina-supplementation significantly decreased aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values compared with control suggesting that spirulina is an appropriate growth-stimulating additive in Nile Tilapia culture.

**Key words:** *Arthrospira fusiformis*, biomass, feed additives, growth parameters, Nile Tilapia.

## INTRODUCTION

The demand for animal protein for human consumption is currently on the rise and is largely supplied with terrestrial farm animals. Aquaculture, however, is an increasingly important option in animal protein production (Fuller, 1992). Fish is an important component of the Egyptian diet. Tilapia is an important food fish in many tropical

areas of Africa, America and Asia. Many species of Tilapia have been cultured in developing countries, where animal protein is lacking. Tilapias are considered suitable for culture, because of their high tolerance to adverse environmental conditions, their relatively fast growth and the ease with which they can breed good utilization of artificial diets, resistance to disease, excellent quality of its firmly textured flesh and finely appetizing fish to consumers (Corpei, 2001). Nile Tilapia, *Oreochromis niloticus* L. is by far the most important farmed Tilapia species. Tilapia is the most familiar and popular fishes in

\*Corresponding author. E-mail: [elsayedb@yahoo.com](mailto:elsayedb@yahoo.com). Tel/Fax: 0020479102930.

Egypt, as well as, in the Middle East and warm climate countries (Philippart and Ruwet, 1982; El-Sherif and El-Feky, 2009). Fish production should be increased in Egypt to meet the demand of the increasing population. Nile Tilapia is an important food fish that has been introduced to many different parts of the world by man. It can today be found on all continents except Antarctica. In several countries, Nile Tilapia has become a problematic invasive species after its introduction.

Microbes defined feed additives play an important and critical roles in aquaculture systems (Irianto and Austin 2002; Kesarcodi-Watson et al., 2008; Belal and Khalafalla 2011). Influences of feed additives on immune responses and bacterial loading in aquatic organisms and environments are well documented (Gatesoupe 1999; Verschuere et al., 2000). As feed additives, dried algae improve growth, feed efficiency, carcass quality, and physiological response to stress and disease in several species of fish (Mustafa and Nakagawa 1995).

Spirulina, *Arthrospira fusiformis* is considered as a rich source of protein, vitamins, minerals, essential amino acids, and fatty acids [gamma-linolenic acid (GLA)], antioxidant pigments such as carotenoids and vitamin E and trace elements (Belay et al., 1996). In addition, it is effective as an immunomodulator (Takeuchi et al., 2002). Several studies have been conducted using dried spirulina as a feed supplement (Watanabe et al., 1990; Ungsethaphand et al., 2010; Ahmadzade-Nia et al., 2011; Mukherjee et al., 2011; Roy et al., 2011). Therefore, the aim of this work was designed to isolate and characterize spirulina, *A. fusiformis*, and using it as a growth promoter in diets for Nile Tilapia (*O. niloticus*).

## MATERIALS AND METHODS

### Isolation of *Arthrospira fusiformis*

Samples of sea water were collected from various niches of a rock pool of a rocky shore, Alexandria and Kafr El-Sheikh (Baltiem city) Governorates, Egypt. Enrichment cultures of spirulina were established from sea water samples on liquid Zarrouk's medium (pH 9) (Pnadey et al., 2010a) supplemented with antibiotics (nystatin (100 mgml<sup>-1</sup>) and cycloheximide (100 mgml<sup>-1</sup>) at 35°C for 20 days and illuminated with day-light fluorescent tubes having 5 Klux at the surface of the vessels. Cultures were manually stirred twice for a few minutes every day. This procedure was repeated four times. Isolation was done using serial dilution and streaking plate method (Ferris and Hirsch, 1991). Samples were diluted with sterilized medium up to 10<sup>-8</sup> dilution. Dilution tubes were incubated under constant light at 35°C. The culture was inoculated into nutrient agar medium and incubated in dark for 3 days (Schwartz et al., 1990). The isolated strains were identified according to Komarek and Anagnostidis (1989), Vonshak and Tomaselli (2000) and Bano and Siddiqui (2004).

### Cyanobacterial growth experiment

*A. fusiformis* with fast growth, highest chlorophyll and protein under laboratory conditions was selected to study the effect of variable media, pH and temperature on its growth. Five media were included

in this study (Pandey et al., 2010a) such as Zarrouk's medium, Rao's media (Singh, 2006), CFTRI media (Venkataraman et al., 1995), OFERR media (Singh 2006), Revised media (6) (Raouf et al., 2006) and Bangladesh medium No.3 (Khatun et al., 1994 and 2006). 500 ml each medium in 1000 ml flask was prepared and the same amount of inoculum in each medium was inoculated. Each flask containing 500 ml from each medium was inoculated with 20 ml of homogenized culture of *A. fusiformis* (10<sup>7</sup> cfu/ml). The pH of Zarrouk's medium (Pandey et al., 2010a) was adjusted to 7, 8, 9, 10, 11 and 12 at 35°C for 25 days and illuminated with day-light fluorescent tubes having 5 Klux at the surface of the vessels. To determine the effect of temperature, Zarrouk's medium with pH of 9 was incubated at 15, 20, 25, 30, 35, 40 and 45°C. The cultures were illuminated with day-light fluorescent tubes having 5 Klux at the surface of the vessels. Cultures were manually stirred twice for a few minutes every day. The experiments were run in triplicates.

### Analytical methods

Biomass concentration (g<sup>-1</sup>) was calculated by measuring dry weight. For dry weight measurement homogenous suspensions of known quantity of spirulina sample were filtered through filter paper 8 mm pore size (Screen printing paper) and oven dried at 75°C for 4 to 6 h. The dried filter paper containing spirulina biomass were cooled and weighed. The difference between the initial and final weight were taken as the dry weight of spirulina biomass. The dry weights were expressed in terms of g<sup>-1</sup>. Samples were taken in triplicates. Chlorophyll-a content was estimated by the method Mackinney (1941). Protein was determined by the method Lowry et al. (1951).

### Experimental fish

Nile Tilapia (*O. niloticus*) fingerlings were brought from a fresh water commercial farm in Motobas, Kafr El-Sheikh Governorate, Egypt. Prior to the start of the experiment, fish were kept in a fiberglass tank and randomly distributed into glass aquaria to be adapted to the experimental condition until the experiment was started. Fish were fed on the control diet for 2 weeks; during this period, healthy fish at the same weight replaced died ones. All the experimental treatments were conducted under an artificial photo period equal to natural light/darkness period (12 h light: 12 h darkness).

### Experimental diets

Three diets were formulated containing feed additives for Nile Tilapia fingerlings: one as a control diet without supplements; second, a supplement at 1% (w/w) with *A. fusiformis*; and third, a supplement at 1% (w/w) with *Spirulina pacifica* (*Arthrospira platensis*, was obtained from Kailua-kona, Hawaii, USA). The basal and tested diets were formulated from the commercial feed ingredients. The dry ingredients were grounded through a feed grinder to a very small size (0.15 mm).

Experimental diets were formulated (Table 1) to be isocaloric and isonitrogenous (31.49% crude protein and 461.63 kcal GE /100 g diet). The ingredients were weighted and mixed by a dough mixer for 20 min to homogenize the ingredients. The estimated amount of oil components (sunflower oil) was gradually added (few drops gradually) and the mixing operation was continued for 20 min. The diets were pelleted through fodder machine and the pellets were dried under room temperature. The diets were collected, and stored in plastic bags in refrigerator at 4°C for further use during the experimental period.

**Table 1.** Composition and proximate analysis of the experimental diets.

Composition (%)	Treatments <sup>5</sup> No. (On DM basis, %)		
	Control (T <sub>1</sub> )	<i>A. fusiformis</i> (T <sub>2</sub> )	<i>S. pacifica</i> (T <sub>3</sub> )
<b>Feed ingredients</b>			
Herring fish meal	15	15	15
Soybean meal	35	35	35
Yellow corn	30	30	30
Wheat bran	15	14	14
Sunflower oil	3	3	3
Vitamins and minerals premix <sup>1</sup>	2	2	2
<i>A. fusiformis</i>	-	1	-
<i>S. pacifica</i>	-	-	1
Total	100	100	100
Dry matter	91.25	91.20	90.89
Crude protein	31.38	31.46	31.58
Ether extract	6.05	6.06	6.04
Crude fiber	5.03	4.10	4.25
Total ash	4.49	4.46	4.43
Nitrogen free extract	53.05	53.90	53.68
<b>Calculated energy value</b>			
GE (kcal/kg) <sup>2</sup>	4583	4625	4620
DE (kcal/kg) <sup>3</sup>	3437	3469	3465
P/E (mg/kcal) <sup>4</sup>	91.30	90.67	91.14

<sup>1</sup>Vitamins and minerals premix at 2% of the diet supplies the following per kg of the diet: 75000 IU Vitamin A; 9000 IU Vitamin D3; 150 mg Vitamin E; 30 mg Vitamin K3; 26.7 mg Vitamin B1; 30 mg Vitamin B2; 24.7 mg Vitamin B6; 75 mg Vitamin B12; 225 mg Nicotinic acid; 69 mg Pantothenic acid; 7.5 mg Folic acid; 150 mg Vit.C; 150 Biotin; 500 mg Choline chlorid 300 mg DL-methionine; 93 mg Fe; 11.25 mg Cu; 210 mg Zn; 204 mg Mn; 5 mg Se and Co 5 mg ( Local market ). <sup>2</sup>GE (Gross energy) was calculated according to NRC (1993) by using factors of 5.65, 9.45 and 4.22 K cal/ggram of protein, lipid and carbohydrate, respectively. <sup>3</sup>DE (Digestible energy) was calculated by applying the coefficient of 0.75 to convert gross energy to digestible energy according to Hephher et al. (1983). <sup>4</sup>P/E (protein energy ratio) = crude protein × 10000 / digestible energy, according to Hephher et al. (1983). <sup>5</sup>Treatments: T<sub>1</sub> (control), without supplements; T<sub>2</sub>, 1% *A. fusiformis*; T<sub>3</sub>, 1% *S. pacifica*.

### Experimental design of rearing fish

A total of 120 Nile Tilapia fingerlings with an average initial body weight of about 7.08 g ± 0.08 were randomly divided into three treatment groups and stocked into 12 glass aquaria (70 L each). Three aquaria were assigned for each treatment. Fresh tap water was stored in fiberglass tanks for 24 h under aeration for dechlorination. One third of all aquaria were replaced daily. Five air stones were used for aerating the aquaria water. Water temperature ranged between 26 to 28°C. Fish feces and feed residues were removed daily by siphoning. Fish from each replicate were weighted at the start of each experiment and hence was counted and weighted every 2 weeks throughout the experimental period (12 weeks).

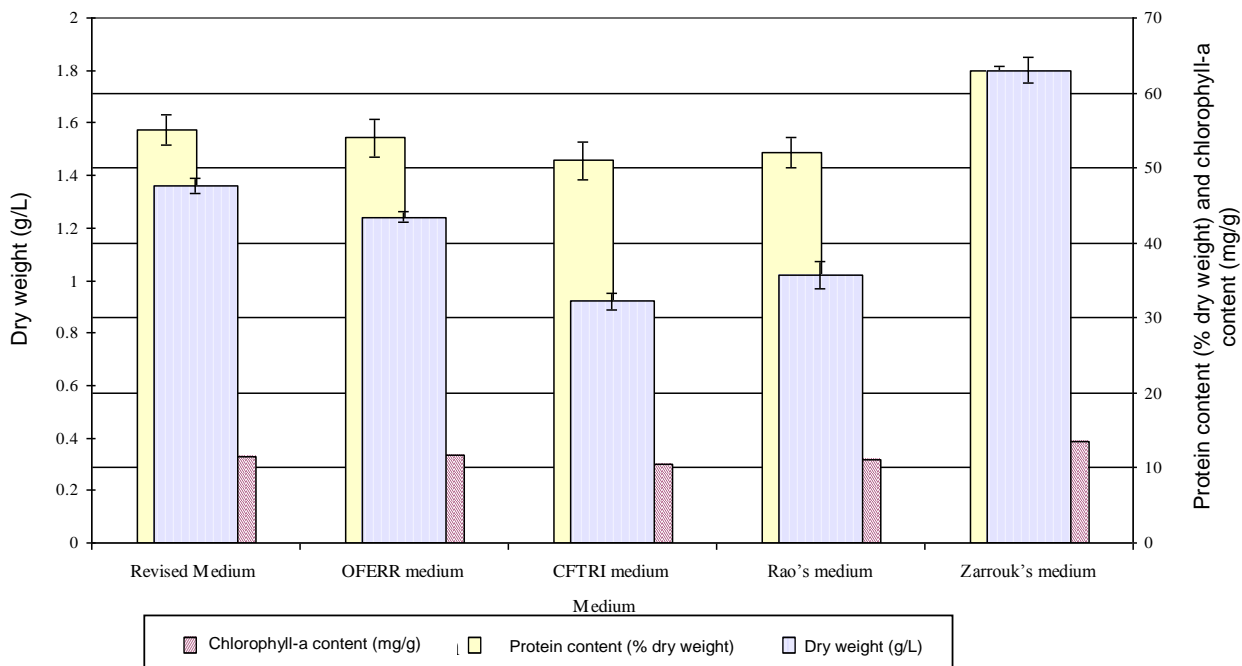
Fish in all treatment were daily fed with the experimental diets at a level of 5, 4 and 3% of the body weight daily for the 1 to 4, 5 to 8 and 9 to 12 weeks, respectively. The feed amount was given three times daily (900, 1200 and 1500) in equal proportions for 12 weeks. Fish were weighed biweekly and feed amounts were adjusted on the basis of the new weight.

### Chemical analysis

Proximate chemical analyses were made of diet ingredients and a sample of fish at the beginning and at the end of the experiment according to standard methods (AOAC 1992) for dry matter, crude protein, ether extract, crude fiber and ash. Gross energy (GE) contents of the experimental diets and fish samples were calculated by using factors of 5.65, 9.45 and 4.22 kcal/g of protein, lipid and carbohydrates, respectively (NRC, 1993).

### Water quality measurements

Water samples were taken each week for monitoring water quality. Analytical methods were done according to the American Public Health Association (APHA, 1985). The pH values were determined by a digital pH-meter, Jenway, model, 3010. Water temperature and oxygen level were measured daily at 8 o'clock by Oxygen meter model 9070. In all treatments, water quality parameters ranged between 26 to 28°C, 7.5 to 8.0, 5.60 to 6.50 mg/L and 0.08



**Figure 1.** Effect of different medium on biomass, chlorophyll- a and protein content production of *A. fusiformis*.

to 0.13 mg/L, for water temperature, pH, dissolved oxygen and water ammonia, respectively. All the water quality parameters were within the acceptable ranges for fish growth (Boyd, 1984).

#### Blood parameters

Blood samples were collected at the end of experiment; fish in each aquarium were weighted and 5 fish were taken randomly for blood sampling. The blood was collected using heparinized syringes from the caudal vein. Blood samples were centrifuged at 4000 rpm for 20 min to allow separation of plasma which was subjected to determine plasma total protein (Tietz, 1990). Blood plasma total lipids were determined according to the method of McGowan et al. (1983). Glucose concentration was determined according to Trinder (1969). Alanine aminotransferase (ALT) and activity of aspartate aminotransferase (AST) were determined by the methods of Young (1990).

#### Statistical analysis

The obtained numerical data were statistically analyzed using SPSS (1997) for one-way analysis of variance (ANOVA). The significance of difference between means was determined by Duncan's multiple range test ( $P < 0.05$ ) (Duncan, 1955).

## RESULTS AND DISCUSSION

Samples of sea water were used to isolate *Arthrospira* on Zarrouk medium by using enrichment technique and 5 isolates from *Arthrospira* were isolated. A preliminary classification based on the morphology and physiology of the isolates revealed that, the 5<sup>th</sup> isolates were identified

as *A. fusiformis* (*Spirulina platensis*, formerly). Enrichment is the process of providing a suitable environment in highly alkaline lakes with high pH for the growth and reproduction of a special group of microalgae while being inhibitory or lethal for non-target organisms. *Spirulina* sp. is photoautotrophic endowed with oxygenic photosynthesis and grows profusely in alkaline lakes of subtropical regions (Vonshak and Tomaselli, 2000). One (1) out of 5 *Arthrospira* strains gave the highest biomass dry weight, chlorophyll and protein content, comparing with the other strains. This strain was selected for the subsequent studies with the aim to evaluate efficacy of different medium, pH and temperature on its biomass dry weight, chlorophyll and protein content.

Result in Figure 1 shows that the dry weight of biomass, chlorophyll-a content and protein content of *A. fusiformis* were higher on Zarrouk medium than the other media (Rao's medium, CFTRI, OFERR and Revised medium 6). The similar studies were done by Pandey et al. (2010a).

Results in Figure 2 exhibited that, *A. fusiformis* was grown at different pH (7, 8, 9, 10, 11, and 12) in flask culture and monitored and expressed in terms of dry weight. The maximum dry weight was 1.92 g/L at pH 9.0 on the 25<sup>th</sup> day after the inoculation. Earlier results also demonstrated that optimum pH for maximum growth of *S. platensis* (*A. fusiformis*) was from 9 to 9.5 (Pandey et al., 2010b). High alkalinity is mandatory for the growth of *A. fusiformis* and bicarbonate is used to maintain high pH. This is because the pH gradually rises as bicarbonate added to the culture medium is dissolved to produce CO<sub>2</sub>,

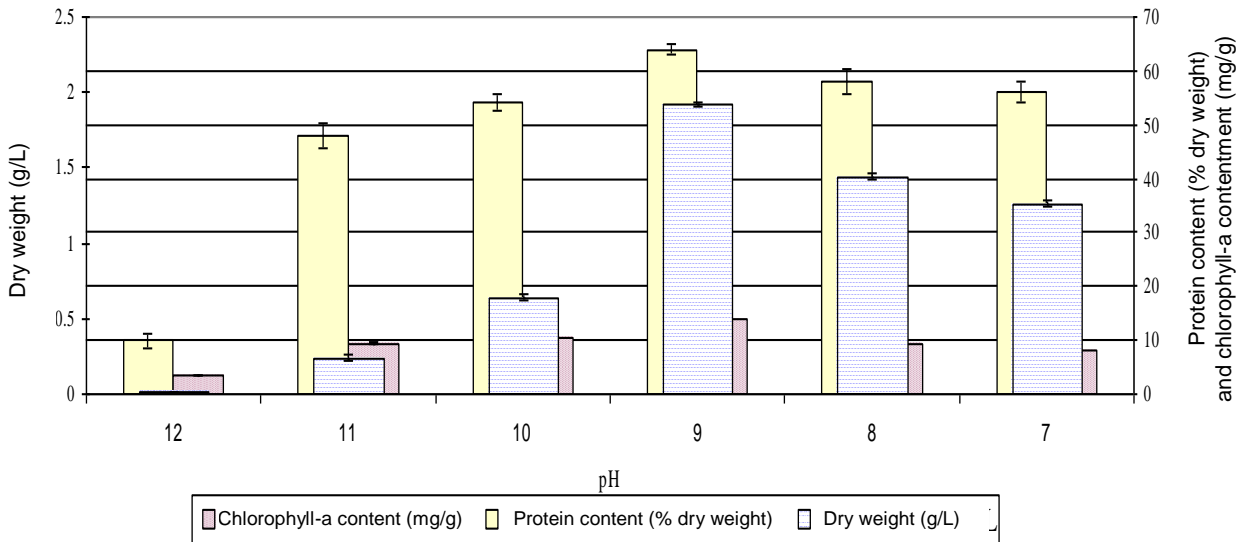


Figure 2. Effect of different medium pH on biomass production of *A. fusiformis*.

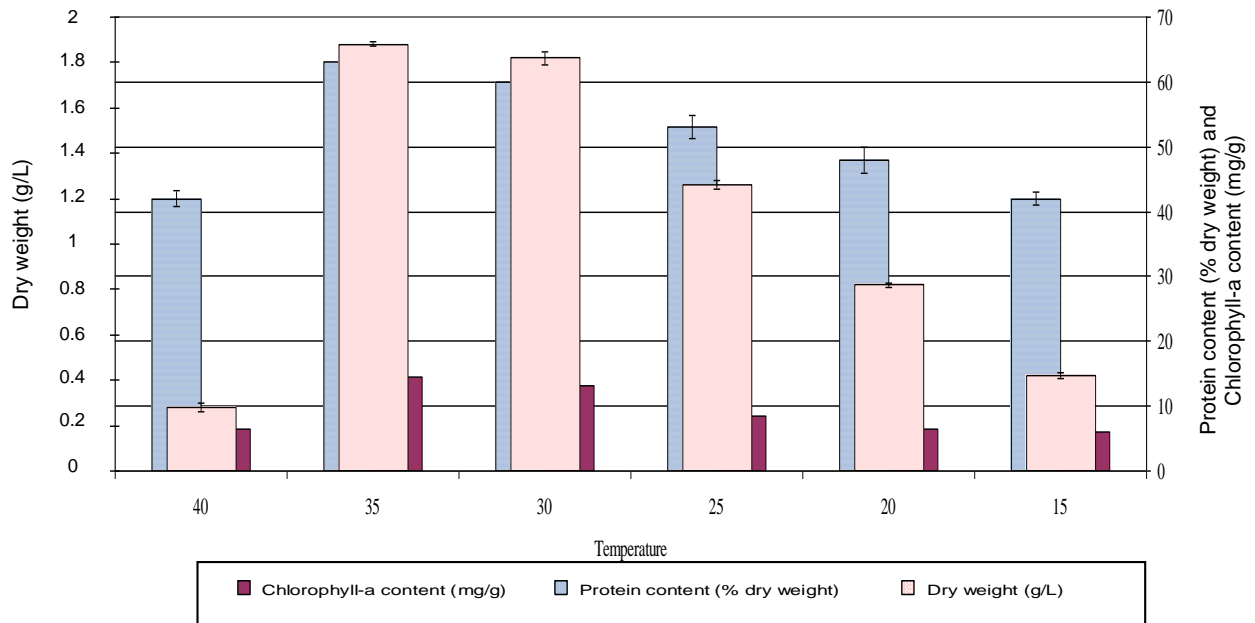


Figure 3. Effect of different temperature on biomass production of *A. fusiformis*.

which releases OH<sup>-</sup> during cultivation of *S. platensis*. *Spirulina* sp. is regarded as obligatory alkalophiles, with the maximal growth rate being obtained at pH 9.5 to 9.8. The fact that it thrives in the high pH environment, which makes life for other microorganisms rather difficult, is the key for the success of large-scale monoalgal cultures of *Spirulina* (Grant et al., 1990). Chlorophyll-a content and protein content are also maximal at pH 9. The Chlorophyll content is 14 mg/g and protein content is 64% of dry

weight. Similar studies have also been done by various workers of cyanobacteria (Kim et al., 2007).

The effect of different temperatures on the growth *A. fusiformis* strain is shown in Figure 3. The growth of *A. fusiformis* was maximal at 35°C. The temperature 35°C appears to be the optimum degree for *A. fusiformis*. Moreover, the tested microbial isolates exhibited growth at 15, 20, 25, 30 and 40°C. No growth was observed at 45°C. It has been shown by previous workers (Pandey et

**Table 2.** Growth performance parameters of Nile Tilapia (*O. niloticus*) fed on the experimental diets.

Item	Treatments			SE*
	Control (T <sub>1</sub> )	<i>A. fusiformis</i> (T <sub>2</sub> )	<i>S. pacifica</i> (T <sub>3</sub> )	
Initial weight (g/fish)	7.10	7.08	7.07	0.08
Final weight (g/fish)	34.21 <sup>b</sup>	39.52 <sup>a</sup>	37.19 <sup>ab</sup>	0.68
Average total gain <sup>1</sup> (g/fish)	27.11 <sup>b</sup>	32.44 <sup>a</sup>	30.12 <sup>ab</sup>	1.21
Average daily gain <sup>2</sup> (g/fish/day)	0.32 <sup>b</sup>	0.39 <sup>a</sup>	0.36 <sup>ab</sup>	0.03
Specific growth rate <sup>3</sup> (SGR %/day)	1.87 <sup>b</sup>	2.05 <sup>a</sup>	1.98 <sup>ab</sup>	0.08
Survival rate <sup>4</sup> (%)	100	100	100	0.001
Feed intake (FI, g/fish)	44.52	45.23	44.61	1.74
Feed conversion ratio <sup>5</sup> (FCR)	1.50 <sup>a</sup>	1.27 <sup>c</sup>	1.35 <sup>b</sup>	0.15
Protein efficiency ratio <sup>6</sup> (PER)	2.13 <sup>c</sup>	2.50 <sup>a</sup>	2.35 <sup>b</sup>	0.14
Protein productive value <sup>7</sup> (PPV, %)	37.49 <sup>b</sup>	45.15 <sup>a</sup>	42.62 <sup>a</sup>	1.38
Energy retention <sup>8</sup> (ER, %)	24.05 <sup>b</sup>	27.62 <sup>a</sup>	26.47 <sup>a</sup>	1.54

Means in the same rows having different superscript letters were significantly different at 0.05 levels. \*Standard error of the mean derived from the analysis of variance. ATG (g/fish) = Average final weight (g) – Average initial weight (g). ADG (g/fish/day) = [ATG (g) / experimental period (d)]. SGR (%/day) = 100 (Ln final weight – Ln initial weight) / experimental period (d). SR = 100 [Total No. of fish at the end of the experimental / Total No. of fish at the start of the experiment]. FCR = DM Feed Intake (g)/Live weight gain (g). PER = Live weight gain (g)/ Protein intake (g). PPV (%) = 100 [Final fish body protein (g) – Initial fish body protein (g)]/crude protein intake (g). ER % = 100 (GE gain / GE intake)

al., 2010) that the optimal growth temperature for *A. fusiformis* is between 30 and 35°C or in the range of 35 to 38°C (Vonshak and Tomaselli, 2000).

*S. platensis* is a cyanobacterium that has been largely studied due to its commercial importance as a source of protein, vitamins, essential amino acids, and fatty acids in many countries in tropic, subtropical and temperate regions for use in human health food, as an animal feed additive (Belay et al., 1996).

*A. fusiformis* was grown under optimum growth conditions and the produced dried biomass was used in the following investigation in feed additives as growth promoters in Nile Tilapia fingerlings diets.

### Chemical composition of diets

Experimental diets (Table 1) contained nearly similar levels of DM, CP, EE, CF, ash, NFE, GE, DE and P/E ratio. The CP and GE content of experimental diets were around 31.49% and 4.62 kcal/g, respectively. These values were within the range suggested for Tilapia by Jauncey and Ross (1982) and NRC (1993).

### Growth performance and survival rate

Data in Table 2 shows the growth performance and nutrient efficiencies on Nile Tilapia fingerlings fed diets. No significant differences in initial body weight were found among the different experimental treatments, indicating the accuracy of randomization process between the experimental treatments.

It is clearly shown (Table 2) that all the tested growth parameters (gain, ADG and SGR) in the diets supplemented with *A. fusiformis* administered to the fingerlings produced the best growth rate. On the other hand, the group of fish fed with control diet exhibited the lowest final body weight. Statistical analysis showed that, the group of fish fed diets supplemented with 1% of *A. fusiformis* and *S. pacifica* had significantly higher values than those control diet.

Results show that, treatments with *A. fusiformis* and *S. pacifica* had feed conversion ratios (FCR) significantly lower than those for the control diets. The best conversion ratio was recorded for the 1% of *A. fusiformis* (1.27) treatments.

In general, fish fed with the diets supplemented with feed additives showed better feeding efficiency than those fed with control diet. The protein efficiency ratio (PER), protein productive value (PPV %) and energy retention (ER %) were significantly higher in the treatments containing 1% *A. fusiformis* and *S. pacifica* than in control treatments. The lowest PER, PPV and ER were recorded in the control treatments.

The best FCR values observed with feed additives - supplemented diets suggest that addition of spirulina improved feed utilization. Similar results have been reported for feed additives used in diets for piglets (Gil, 1998). In practical terms, this means that feed additives use can decrease the amount of feed necessary for animal growth which could result in production cost reductions.

The PER and PPV results indicate that supplementing diets with spirulina significantly improves protein utilization in Tilapia. This contributes to optimizing protein

**Table 3.** Effect of *A. fusiformis* and *S. pacifica* on Nile Tilapia body composition (% on DM basis).

Item	Initial fish	Treatments			SE*
		Control (T <sub>1</sub> )	<i>A. fusiformis</i> (T <sub>2</sub> )	<i>S. pacifica</i> (T <sub>3</sub> )	
Dry matter, (%)	23.72	28.52	28.64	28.90	0.29
Crude protein (%)	57.10	58.84 <sup>b</sup>	60.21 <sup>a</sup>	59.68 <sup>a</sup>	1.42
Ether extract (%)	17.65	20.24 <sup>a</sup>	19.58 <sup>b</sup>	19.89 <sup>b</sup>	0.56
Ash (%)	14.21	14.42 <sup>b</sup>	15.53 <sup>a</sup>	15.80 <sup>a</sup>	0.10
Energy, (kcal/100 g)	536	551	545	545	2.54

Means in the same rows having different superscript letters were significantly different at 0.05 levels. \*Means of the standard error derived from the analysis of variance.

**Table 4.** Blood plasma parameters of Nile Tilapia fed on the experimental diets.

Item	Treatments			SE*
	Control (T <sub>1</sub> )	<i>A. fusiformis</i> (T <sub>2</sub> )	<i>S. pacifica</i> (T <sub>3</sub> )	
Plasma glucose (mg/dl)	58.11 <sup>b</sup>	62.54 <sup>a</sup>	63.87 <sup>a</sup>	0.45
Plasma total protein (g/dl)	5.10 <sup>b</sup>	7.58 <sup>a</sup>	6.97 <sup>a</sup>	0.15
Plasma total lipid (g/dl)	4.23 <sup>b</sup>	5.28 <sup>a</sup>	4.89 <sup>a</sup>	0.12
AST (U/dl)	115 <sup>a</sup>	108 <sup>b</sup>	112 <sup>b</sup>	3.45
ALT (U/dl)	46 <sup>a</sup>	42 <sup>b</sup>	41 <sup>b</sup>	1.58

Means in the same rows having different superscript letters were significantly different at 0.05 levels. \*Standard error of the mean derived from the analysis of variance.

use for growth, a significant quality given that protein is the most expensive feed nutrient. The improvement in the biological value of the supplemented diets in these treatments with high population and low dietary protein demonstrated that spirulina supplements performed more efficiently in stress situations (Ringo and Gatesoupe, 1998).

### Body composition

Feed additives supplementation significantly affected whole-fish body composition except for dry matter, which did not differ (Table 3). Fish fed with the control diet had the lowest protein content; however, all feed additives supplementation appeared to improved protein content without significant difference. Carcass lipid content was also affected by dietary protein content, with the highest values in the control treatment, which were statistically different from the supplemented treatments. The lowest overall lipid content was recorded with *A. fusiformis* treatment, which was not statistically different with all other treatments. Ash content increased significantly with the increase of feed additives and the highest ash content was obtained in fish fed with 1% *S. pacifica*, whereas the lowest was obtained in fish fed with the control diet.

These results suggest that *A. fusiformis* supplementation plays a role in enhancing feed intake with a subsequent enhancement of fish body composition. The

better feed intake in spirulina- supplemented diets may have been due to increase fish appetite resulting in a higher feed intake and therefore improved growth. Moreover, due to the high feed intake and nutrient utilization, the deposited nutrients increased. On the other hand, changes in protein and lipid content in fish body could be linked with changes in their synthesis, deposition rate in muscle and/or different growth rate (Soivio et al., 1989; Abdel-Tawwab et al., 2006).

### Biochemical blood parameters

Results in Table 4 showed that, fish fed with diets containing feed additives exhibited higher glucose, lipid and protein values. Also, feed additives supplementation significantly decreased AST and ALT values compared with control.

Biochemical analyses often provide vital information for health assessment and management of cultured fish (Pincus, 1996; Cnaani et al., 2004; Řehulka et al., 2004). In the present study, fish fed with diets containing 1% spirulina exhibited higher glucose, lipid and protein values. These results suggest an improvement of fish health when fed with feed additives supplement. Moreover, the measurement of AST and ALT in plasma is of considerable diagnostic value in fish, as it relates to general nutritional status as well as the integrity of the vascular system and liver function. This result agrees with Abdel-Tawwab et al. (2008) who investigated the

use of commercial baker yeast as a growth and immunity promoter for Nile Tilapia; they found that biochemical parameters were improved in fish fed yeast.

## Conclusion

It can be concluded that the addition of 1% spirulina in Tilapia fingerlings diets improves animal growth, and mitigates the effects of stress factors. The two tested strains used in the present study were effective in stimulating fish performance, though *A. fusiformis* produced the best results, being the most viable option for optimizing growth and feed utilization in intensive Tilapia culture.

## REFERENCES

- AOAC (1992). Official Methods of Analysis of the Association of Official Analytical Chemists, 14th ed. AOAC, Arlington. p. 3413.
- Abdel-Tawwab ME, Khattab YA, Ahmad MH, Shalaby AME (2006). Compensatory growth, feed utilization, whole-body composition and hematological changes in starved juvenile Nile tilapia, *Oreochromis niloticus* (L.). J. Appl. Aquac. 18:17–36.
- Abdel-Tawwab M, Abdel-Rahman AM, Ismael NEM (2008). Evaluation of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for Fry Nile tilapia, *Oreochromis niloticus* (L.) challenged in situ with *Aeromonas hydrophila*. Aquaculture 280:185–189.
- Ahmadzade-Nia Y, Adl KN, Hezave SG, Hejazi MA, Hassanpour S, Chaichisemari M, Riyazi SR (2011). Effect of replacing different levels of Soybean meal with *Spirulina* on performance in Rainbow Trout. Ann. Biol. Res. 2 (3):374-379.
- APHA, American Public Health Association (1985). Standard methods for the examination of water and waste. 12th addition, Inc. New York. p. 769.
- Bano A, Siddiqui PJA (2004). characterization of five marine cyanobacterial species with respect to their pH and salinity requirements. Pak. J. Bot. 36(1):133-143.
- Belal EB, Khalafalla MME (2011). Biodegradation of *Panicum repens* residues by *Pleurotus ostreatus* for its use as a non conventional feedstuff in diets of *Oreochromis niloticus*. Afr. J. Microbiol. Res. 5(19):3038-3050.
- Belay A, Kato T, Ota Y (1996). *Spirulina* (*Arthrospira*): potential application as an animal feed supplement. J. Appl. Phyco. 8:303-311.
- Boyd CE (1984). Water Quality in Warm water Fishponds. Auburn University Agriculture Experimental Station, Auburn, AL, USA (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*). Aquaculture 161:27–43.
- Cnaani A, Tinman S, Avidar Y, Ron M, Hulata G (2004). Comparative study of biochemical parameters in response to stress in *Oreochromis aureus*, *O. mossambicus* and two strains of *O. niloticus*. Aquac. Res. 35:1434–1440.
- Corpei A (2001). Product Profile Tilapia. Expansion of Ecuador's Export Commodities, CBI Project.
- Duncan MB (1955). Multiple ranges and multiple F-tests. Biometrics 11:1-42.
- El-Sherif MS, El-feky AMI (2009). Performance of Nile Tilapia (*Oreochromis niloticus*) Fingerlings .I. Effect of pH. Int. J. Agric. Biol. 11:297–300.
- Ferris MJ, Hirsch CF (1991). Method for Isolation and Purification of Cyanobacteria. Appl. Environ. Microbiol. pp. 1448-1452.
- Fuller R (1992). Feed additives. The Scientific basis. Chapman and Hall, London, UK, p. 398.
- Gatesoupe FJ (1999). The use of feed additives in aquaculture. Aquaculture 180:147–165.
- Gil F (1998). Empleo de enzimas en nutricio'n animal. <http://www.ered.districto.com/usuarios/Pacogil/enzimas.htm>, pp.1-4.
- Grant WD, Mwatha WE, Jones BE (1990). Alkaliphiles: ecology, diversity and application. FEMS Microbiol. Rev. 75:225-270.
- Hepher B, Liao IC, Cheng SH, Haseih CS (1983). Food utilization by red tilapia. Effect of diet composition, feeding level and temperature on utilization efficiency for maintenance and growth. Aquaculture 32:255-272.
- Irianto A, Austin B (2002). Use of feed additives to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish. Dis. 25:333–342.
- Jauncey K, Ross B (1982). A guide to tilapia feeds and feeding. In: Aquaculture, Univ. Sterling, FK94 La, Scotland, U.K. p. 111.
- Kesarcodi-Watson A, Kaspar H, Josie LM, Gibson L (2008). Feed additives in aquaculture: the need, principles and mechanisms of action and screening processes. Aquaculture 274:1–8.
- Kim CJ, Jung YH, OH HM (2007). Factors indicating culture status during cultivation of *Spirulina* (*Arthrospira*) *platensis*. J. Microbiol. 45(2):122-127.
- Khatun R, Hossain MM, Begum SMS, Majid FZ (1994). *Spirulina* culture in Bangladesh V. Development of simple, inexpensive culture media suitable for rural or domestic level cultivation of *Spirulina* in Bangladesh. J. Sci. Ind. Res. 29:163-166.
- Khatun R, Noor P, Akhter N, Jahan MAA, Hossain M, Munshi JL (2006). *Spirulina* Culture in Bangladesh XI Selection of a Culture Medium, Suitable for Culturing a Local Strain of *Spirulina*. Bangladesh J. Sci. Ind. Res. 41(3-4):227-234.
- Komarek J, Anagnostidis K (1989). Modern approach to the classification system of cyanophytes; 4-Nostocales. Arch. Hydrobiol. 83, Algol. Stud. 56:247-345.
- Lowry OH, Rosebrough NL, Farr AL, Radall RJ (1951). Protein measurement with the folin-phenol reagent. J. Biol. Chem. 193:265-275.
- Mackinney G (1941). Absorption of light by chlorophyll solution. J. Biol. Chem. 140:466-469.
- McGowan MW, Artiss JD, Standbergh DR, Zak BA (1983). Peroxidase-coupled method for colorimetric determination of serum triglycerides. Clin. Chem. 29:538.
- Mukherjee S, Parial D, Khatoon N, Chaudhuri A, Senroy S, Homechaudhuri S, Pal R (2011). Effect of Formulated Algal Diet on growth performance of *Labeo rohita* Hamilton. J. Algal. Biomass Utln. 2(4):1-9.
- Mustafa MG, Nakagawa YH (1995). A review: Dietary benefits of algae as an additive in fish feed. Isreal J. Aquacult. 47:155-162.
- NRC (1993). Nutrition requirements of fish. National Research Council National academy press, Washington, D. C. USA. p. 114.
- Pandey JP, Tiwari A, Mishra RM (2010a). Evaluation of Biomass Production of *Spirulina maxima* on Different Reported Media. J. Algal Biomass Utln. 1(3):70-81.
- Pandey JP, Pathak N, Tiwari A (2010b). Standardization of pH and Light Intensity for the Biomass Production of *Spirulina platensis*. J. Algal Biomass Utln. 2,1(2):93-102.
- Philippart JCL, Ruwet JCL (1982). Ecology and distribution of tilapias. In: R. H. Lowe-Mc Connell (eds.). The Biology and Culture of Tilapia. International Center for living Aquatic Resources Management, Manila, Philippines. pp. 15-59.
- Pincus MR (1996). Interpreting laboratory results: reference values and decision making, In: Henry, J.B. (Ed.), Clinical Diagnosis and Management by Laboratory Methods, Nineteenth edition. W.B. Saunders, Philadelphia. PA. USA. pp. 74-91.
- Raof B, Kaushik BD, Prasanna R (2006). Formulation of a low-cost medium for mass production of *Spirulina*. Biom. Bioen. 30(6):537-542.
- Řehulka J, Minařík B, Řehulková E (2004). Red blood cell indices of rainbow trout *Oncorhynchus mykiss* (Walbaum) in aquaculture. Aquacult. Res. 35:529-546.
- Ringo E, Gatesoupe FJ (1998). Lactic acid bacteria in fish: a review. Aquaculture 160:177-203.
- Roy SS, Chaudhuri A, Mukherjee S, Chauduri SH, Pal R (2011). Composite algal supplementation in nutrition of *Oreochromis mossambicus*. J. Algal. Biomass. Utln. 2 (1):10-20.
- Schwartz RE., Hirsch CF, Sesin DF, Flor JE, Chartrain M, Fromtling RE, Harris GH, Salvatore MJ, Liesch JM, Yudin K (1990).



- Pharmaceuticals from cultured algae. *J. Ind. Microbiol.* 5:113-124.
- Singh S (2006). *Spirulina*: A Green gold mine. Paper presented at: Spirutech 2006. *Spirulina* cultivation: Potentials and Prospects. Jabalpur, Madhya Pradesh.
- Soivio A, Niemisto M, Backstrom M (1989). Fatty acid composition of *Coregonus muksun* Pallas: changes during incubation, hatching, feeding and starvation. *Aquaculture* 79:163-168.
- SPSS (1997). Statistical package for the social sciences, Versions 6, SPSS in Ch, Chi-USA.
- Takeuchi T, Lu J, Yoshizaki G, Satoh YS (2002). Effect on the growth and body composition of uvenile tilapia *Oreochromis niloticus* fed raw *Spirulina*. *Fish. Sci.* 68:34-40.
- Tietz NW (1990). *Clinical Guide to Laboratory Tests* 2nd Ed. Philadelphia. Tovar D, Zambonino-Infante JL, Cahu C, Gatesoupe FJ, Vázquez-Juárez R, Lésel R (2002). Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass larvae. *Aquaculture* 204:113-123.
- Trinder B (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. *Ann. Clin. Biochem.* 6:24-32.
- Ungsethaphand T, Peerapornpisal Y, Whangchai N, Sardud U (2010). Effect of feeding *Spirulina platensis* on growth and carcass composition of hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*). *Maejo Int. J. Sci. Technol.* 4(02): 331-336.
- Verschuere L, Rombaut G, Sorgeloos P, Verstraete W (2000). Feed additives bacteria as biological control agents in aquaculture. *Microbiol. Mol. Biol. Rev.* 64:655-671.
- Vonshak A, Tomaselli L (2000). *Arthrospira (Spirulina)*: Systematics and ecophysiology. In: Whitton, A., Potts, M., Eds. *The Ecology of Cyanobacteria*. Kluwer Academic Publishers. The Netherlands. p. 505-522.
- Watanabe T, Liao W, Takeuchi T, Yamamoto YH (1990). Effect of dietary *Spirulina* supplement on growth performance and flesh lipids of cultured striped jack. *J. Tokyo Univ. Fish.* 77:231-239.
- Young DS (1990). *Effects of drugs on clinical laboratory tests*. Third Edition, AACC Press, Washington, D.C. 32(3):30-33.