

USE OF SPIRULINA (*ARTHROSPIR PLATENSIS*) AS A GROWTH AND IMMUNITY PROMOTER FOR NILE TILAPIA, *OREOCHROMIS NILOTICUS* (L.) FRY CHALLENGED WITH PATHOGENIC *AEROMONAS HYDROPHILA*

MOHSEN ABDEL-TAWWAB¹, MOHAMMAD H. AHMAD², YASSER M. ABDEL-HADI³ AND MEDHAT E.A. SEDEN²

1. Fish Ecology Department

2. Fish Nutrition Department

3. Fish Disease Department, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt.

• Corresponding author E-mail address: mohsentawwab@yahoo.com

Abstract

This study was undertaken to evaluate the use of Spirulina (*Arthrospir platensis*) as a growth and immunity promoter for Nile tilapia, *Oreochromis niloticus* (L.). Fish (1.88 g) were randomly distributed into six group each at a rate of 20 fish per 100-L aquarium and fed on a diet containing either 0.0, 1.25, 2.5, 5.0, 7.5, or 10.0 g Spirulina/kg diet for 12 weeks. After the feeding trial, fish of each treatment were challenged by pathogenic *Aeromonas hydrophila*, which was given by intraperitoneal (IP) injection and they were kept under observation for 10 days to record any abnormal clinical signs and the daily mortality rate. The growth-promoting influence of Spirulina was observed with fish. The optimum growth and feed utilization were obtained at 5.0 g Spirulina/kg diet. No significant changes in fish survival among the different treatments, although Spirulina supplementation increased protein deposition in fish body especially when fed on 1.25 – 5.0 g/kg diet. No significant differences in lipid and ash contents were observed among the different treatments. The physiological parameters were improved when fish fed Spirulina supplement. However, the highest red blood cells (RBC), white blood cells (WBC), and nitro blue tetrazolium (NBT) values were obtained at 5.0 - 10.0 g Spirulina/kg diet; meanwhile the lowest values were obtained at control. Total fish mortality 10-days after IP injection with *A. hydrophila* and its count after incubation with fish serum decreased with the increase of Spirulina level in fish diets. The lowest fish mortality and bacterial counts were obtained when fish fed 5.0 - 10.0 g Spirulina/kg. These results indicate that Spirulina supplementation is promising for disease prevention in tilapia culture, and the optimum level of Spirulina in fish diet is 5.0 - 10.0 g per kg diet.

Key words: Spirulina, Nile tilapia, growth performance, feed utilization, body composition, physiological parameters, NBT, non-specific immunity, *Aeromonas hydrophila*.

INTRODUCTION

Proper nutrition has long been recognized as a critical factor in promoting normal growth and sustaining fish health. Prepared diets not only provide the essential nutrients that are required for normal physiological functioning, but also may serve as the medium by which fish receive other components that may affect their health (Gatlin, 2002). However, research on diets optimization to enhance fish health is still in its infancy.

Spirulina, *Arthrospir platensis* is a freshwater blue-green filamentous alga, and it is receiving increasing attention for its bioactive components such as vitamins, minerals, polyunsaturated fatty acids, carotenes and other pigments that have antioxidants activity (Cohen and Vonshak, 1991; Mahajan and Kamat, 1995; Bhat and Madyastha, 2000; Madhava *et al.*, 2000; Lin *et al.*, 2007; Wang *et al.*, 2007). Bermejo *et al.* (2008) reported that most antioxidant capacities of Spirulina protean extract are attributable to the biliproteins contained in this microalga, such as phycocyanin. The previous authors suggested that Spirulina could be used to produce a natural dietary antioxidant supplement or added to healthy food products, such as cereals, fruit bars or drinks, to prevent some chronic diseases where free radicals are involved. Moreover, Spirulina is a rich source of protein (60–70%), vitamins, essential amino acids, minerals, and essential fatty acids such as palmitic acid, linolenic acid, and linoleic acid. Therefore, it has been used as a nutrient for fish larvae (Lu *et al.*, 2002; Lu and Takeuchi, 2004) and as an ingredient in fish diet for juveniles and adults common carp (Nandeeshha *et al.*, 1998, 2001; Takeuchi *et al.*, 2002; Palmegiano *et al.*, 2005, 2008).

Tilapia farming has grown extremely fast in the last decade, where they are cultured worldwide with annual growth rate of about 12.2% (El-Sayed, 2006). Nile tilapia, *Oreochromis niloticus* is one of the most important species within the tilapia species. Abdelghany (1993), Getabu (1994), and Abdel-Tawwab and El-Marakby (2004) notes that Nile tilapia, *O. niloticus* is omnivorous and can utilize a wide range of food items including blue-green algae. Therefore, the present study was conducted to determine the effects of graded levels of Spirulina (*A. plantensis*) on the growth performance, feed utilization, non-specific immune responses, and resistance of Nile tilapia to *Aeromonas hydrophila* infection.

MATERIALS AND METHODS

Algae culture

A pure culture of Spirulina, *A. platensis* (Gomont) Geitler was obtained from Botany Department, Faculty of Science, Zagazig University, Zagazig, Egypt. The basal medium was adopted by Zarrouk (1966). The alga was cultivated in 50 L photobioreactors using a unialgal semi-continuous culture (Morist *et al.*, 2001). To determine algal biomass, a

100 mL aliquot of the algae suspension was filtered through a Millipore filter (0.45 μm), and the obtained floc was then oven-dried at 85 °C for 4 h and weighed.

Fish culture and feeding regime

Nile tilapia, *O. niloticus* (L.) fry were obtained from fish hatchery at the Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. Fish were kept in indoor fiberglass tanks for 2 weeks for acclimation to the laboratory conditions. Fifty fish were frozen at – 20 °C for chemical analysis at initial. Six group of Nile tilapia (1.88 g) were randomly distributed into six group each at a rate of 20 fish per 100-L aquarium (triplicate each treatment). Each aquarium was supplied with compressed air via air-stones using aquarium air pumps. Settled fish wastes were cleaned daily by siphoned with three quarters of the aquarium's water, which was replaced by aerated water from the water storage tank. Water temperature range was 26 - 30 °C. Each diet was fed twice daily; six days a week for a period of 12 weeks. The feeding rate was 10% of live body weight for the first 4 weeks, reduced to be 5% for the rest of the experimental period. Fish in each aquarium were group-weighed every 2 weeks and the amounts of feed given were readjusted accordingly. Dead fish was daily recorded and removed.

Diet preparation

A basal diet was formulated to contain 30.6% crude protein, 9.1% lipids, and 4.72 kcal GE/g diet (Table 1). Corresponding Spirulina culture volume was diluted by distilled water up to 100 ml as a final volume, which was then added to the basal diet to represent the levels of 0.0 (control), 1.25, 2.5, 5.0, 7.5, or 10.0 g/kg diet. Spirulina was suspended in 100 ml distilled water and added to the ingredients of each diet, which was blended for 40 min at least to make a paste of each diet. The pastes were separately passed through a grinder, and pelleted (1 mm diameter) in a paste extruder. The diets were air-dried and stored in plastic bags in a refrigerator (- 2 °C) for further use.

Chemical analysis of diets and fish

The tested diets and whole-fish body from each treatment at the beginning and at the end of experiment were analyzed according to the standard methods of AOAC (1990) for moisture, protein, fat and ash. Moisture content was estimated by drying the samples to constant weight at 85 °C in drying oven (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA). Nitrogen content was measured using a microkjeldahl apparatus Labconco (Labconco Corporation, Kansas, Missouri, USA) and crude protein was estimated by multiplying nitrogen content by 6.25. Lipid content was determined by ether extraction in multi-unit extraction Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA) for 16 hours and ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550 °C for 6 hours.

Table 1. Composition and proximate chemical analyses (%; on dry matter bases) of the experimental diets.

Ingredients	Basal diet	Spirulina
Herring fish meal	9.10	
Soybean flour	52.57	
Corn flour	19.25	
Starch	7.00	
Corn oil	1.80	
Cod liver oil	1.98	
Vitamin premix ⁽¹⁾	2.00	
Mineral premix ⁽²⁾	2.00	
α -cellulose	3.30	
Carboxy-methyl-cellulose	1.0	
Total	100	
Chemical analysis (%)		
Dry matter	91.7	23.2
Crude protein	30.6	62.4
Ether extract	9.1	6.3
Ash	8.3	8.7
Nitrogen free extract ⁽³⁾	52.0	22.6
Gross energy (kcal/g) ⁽⁴⁾	4.72	5.04

¹ Vitamin premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; α -tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

² Mineral premix (per kg of premix): CaHPO₄.2H₂O, 727.2 g; MgCO₄.7H₂O, 127.5 g; KCl 50.0 g; NaCl, 60.0 g; FeC₆H₅O₇.3H₂O, 25.0 g; ZnCO₃, 5.5 g; MnCl₂.4H₂O, 2.5 g; Cu(OAc)₂.H₂O, 0.785 g; CoCl₃.6H₂O, 0.477 g; CaIO₃.6H₂O, 0.295 g; CrCl₃.6H₂O, 0.128 g; AlCl₃.6H₂O, 0.54 g; Na₂SeO₃, 0.03 g.

³ NFE (nitrogen free extract) = 100 – (protein% + lipid% + ash% + fiber %)

⁴ GE (gross energy) was calculated after NRC (1993) as 5.64, 9.44 and 4.11 Kcal/g for protein, lipid and NFE, respectively.

Water analysis

Water samples were collected biweekly at 15 cm depth from each aquarium. Dissolved oxygen and temperature were measured on site with a YSI model 58 oxygen meter (Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). Unionized ammonia was measured using DREL/2 HACH kits (HACH Co., Loveland, Co., USA). The pH was measured using a pH-meter (Digital Mini-pH Meter, model 55, Fisher Scientific, USA). In all treatments, dissolved oxygen concentrations range was 4.9 - 7.2 mg/L, pH range was 7.5 – 7.8, and unionized ammonia concentration range was 0.08 - 0.17 mg/L. All the previous water quality parameters are within the acceptable range for fish growth (Boyd, 1984).

Growth parameters

Growth performance was determined and feed utilization was calculated as following:

Weight gain = final weight – initial weight;

Specific growth rate (SGR) = $100 (\ln W_2 - \ln W_1) / T$; where W_1 and W_2 are the initial and final weight, respectively, and T is the number of days in the feeding period;

Feed conversion ratio (FCR) = feed intake / weight gain;

Protein efficiency ratio (PER) = weight gain / protein intake;

Apparent protein utilization (APU; %) = $100 \times (\text{protein gain} / \text{protein intake})$;

Energy utilization (EU; %) = $100 \times (\text{energy gain} / \text{energy intake})$.

Physiological measurements

At the end of the 12-week feeding trial, fish were fasted for 24 hours immediately prior to blood sampling and five fish per aquaria were randomly chosen and anesthetized with tricaine methanesulfate (20 mg/L). The blood was extracted from the caudal vein and divided in two sets of Eppendorf tubes. One set contained sodium heparinate (500 U/L), used as anticoagulant, for the counting of red blood cells (RBC) and white blood cell (WBC), which were done following the methods of Brown (1988). Blood smears were fixed in methanol and stained with Wright–Giemsa stain for determination of the differential WBC count. At least 200 WBCs were counted for differential WBC determinations. The second set was left with no anticoagulant in order to clot at 4 °C and centrifuged at 5000 rpm for 5 min at room temperature. Glucose was determined calorimetrically according to Trinder (1969). Total protein content in plasma was determined calorimetrically according to Henry (1964). Total lipids content in plasma was determined calorimetrically according to Joseph *et al.* (1972). Albumin

and globulin in plasma were determined calorimetrically according to Wotton and Freeman (1982). The production of oxygen radicals by leukocytes was assayed by the reduction of Nitro Blue Tetrazolium (NBT, Sigma-Aldrich Chemical, St. Louis, MO, USA) according to Rook *et al.* (1985). Absorbance was converted to NBT units based on a standard curve of NBT diformazan per milliliter of blood.

Challenge test

At the end of the study, fish in each aquarium were divided into two groups. The first group was challenged with pathogenic *A. hydrophila*. A 0.1 ml dose of 24-h broth from virulent bacterial pathogen of *A. hydrophila* (5×10^5 cells/ml) was given by intraperitoneal (IP) injection (Schaperclaus *et al.*, 1992). The second group was IP injected by 0.1 ml of saline solution as a control. All groups were kept under observation for 10 days to record any abnormal clinical signs and the daily mortality rate.

Bacteriocidal activity

Bacteriocidal activity in fish samples was analyzed according to the Miles–Misra technique (Okada *et al.*, 1999). *A. hydrophila* used in this assay was stocked at -80 °C in glycerol solution. This strain was inoculated with tryptone soy broth (TSB). Bacteria culture was grown for 24 h at 30 °C on TSB medium, and the culture broth was centrifuged, washed with saline solution and suspended in gelatin veronal buffer (GVB). Bacterial densities were adjusted to 1×10^5 cells/ml using a spectrophotometer and viable counting was conducted by inoculating with serial dilution in GVB on tryptone soy agar (TSA). The serum sample was mixed with GVB (1: 4 v/v) and stored at 4 °C until use. Serum samples were mixed with an equal volume of bacterial suspension and incubated at 30 °C. Viable counts were checked by the agar plate-spread method on TSA 24 h after incubation.

Statistical analysis

The obtained data were subjected to one-way ANOVA to evaluate the effect of Spirulina supplement. Differences between means were tested at the 5% probability level using Duncan Multiple Range test. All the statistical analyses were done using SPSS program version 10 (SPSS, Richmond, VA, USA) as described by Dytham (1999).

RESULTS

Final fish weight, weight gain, and specific growth rate increased significantly ($P < 0.05$) with the increase in Spirulina levels in fish diets (Table 2). The optimum growth was obtained at 5.0 - 10.0 g Spirulina/kg diet, whereas the control diet

produced the lowest fish growth. No significant differences in fish survival among the different treatments ($P > 0.05$), and its range was 93.3 - 95.6%. Moreover, fish fed on diets containing 0.5 g Spirulina/kg consumed more diet than the other fish groups giving the lowest FCR (1.22). Fish fed on control diet consumed less diet giving a higher FCR (1.43). Fish fed 5.0 g Spirulina/kg diet produced the highest PER, APU, and EU (Table 2).

Spirulina supplementation had no significant effect on the whole-fish body composition except protein content, which was significantly affected (Table 3). No significant differences in moisture, lipid and ash contents were observed among the different treatments ($P > 0.05$). Fish fed on 1.25 – 5.0 g Spirulina/kg diet exhibited the highest protein content without significant difference among them ($P > 0.05$).

Fish fed on diets containing 2.5 - 10.0 g Spirulina/kg diet exhibited similar RBCs and WBCs counts and their ranges were $2.07 - 2.54 \times 10^6/\mu\text{L}$ and $3.29 - 4.02 \times 10^5/\mu\text{L}$, respectively ($P < 0.05$; Table 4). The low counts of RBCs and WBCs were obtained at the control diet $1.87 \times 10^6/\mu\text{L}$ and $3.13 \times 10^5/\mu\text{L}$, respectively. No significant changes in lymphocytes were observed at 2.5 – 10.0 g/kg diet ($P < 0.05$), whereas the lowest one was observed at the control diet (84.4%). Contrarily, the control diet produced the highest monocytes and granulocytes, which decreased with the increase of Spirulina levels in fish diet ($P < 0.05$; Table 4).

Serum glucose, lipids, protein, albumin, and globulin values increased with the increase of Spirulina supplementation and the highest values were obtained at 5.0 - 10.0 g Spirulina/kg diet without significant difference among them (Table 5). The lowest values of the above parameters were obtained at the control diet. Similarly, Spirulina supplementation stimulated superoxide anion production (NBT values) and the highest NBT values were obtained at 5.0 - 10.0 g Spirulina/kg diet, meanwhile the lowest value was obtained at the control diet (Table 6). The bacterial count after incubation with fish sera decreased with increase of Spirulina level in fish diets. The lowest bacterial count was obtained in fish fed 5.0 - 10.0 g Spirulina/kg, whereas the highest one was obtained when fish fed control diet (Table 6).

Fish mortality after IP injection with *A. hydrophila* increased significantly ($P < 0.05$) by increasing the rearing time and the maximum fish mortality rate was obtained 4 days post bacterium IP injection after which no mortality was observed (Fig. 1). The total fish mortality 10 days after IP with *A. hydrophila* decreased significantly ($P < 0.05$) with the increase of Spirulina supplementation. The bacterial count after incubation with fish sera decreased with increase of Spirulina level in fish diets. The lowest bacterial count was obtained in fish fed 5.0 - 10.0 g Spirulina/kg, whereas the highest one was obtained when fish fed control diet (Table 6).

Table 2. Growth performance and feed utilization of Nile tilapia fed practical diets containing different levels of Spirulina for 12 weeks.

Items	Spirulina levels (g/kg diet)					
	Control (0.0)	1.25	2.5	5.0	7.5	10.0
Initial weight (g)	1.87±0.015 a	1.89±0.010 a	1.89±0.023 a	1.88±0.024 a	1.88±0.019 a	1.89±0.023 a
Final weight (g)	14.12±0.62 c	14.81±0.27 bc	15.27±0.23 b	16.93±0.16 a	14.68±0.43 c	14.63±0.81 c
Weight gain (g)	12.25±0.61 c	12.92±0.27 bc	13.38±0.24 b	15.05±0.15 a	12.80±0.45 c	12.74±0.78 c
SGR (%/day)	2.41±0.047 c	2.45±0.026 bc	2.49±0.028 b	2.62±0.013 a	2.45±0.047 bc	2.44±0.050 bc
Survival rate (%)	95.6±2.2 a	95.6±2.2 a	93.3±3.8 a	95.6±2.2 a	93.3±3.8 a	95.6±2.2 a
Feed intake (g feed/fish)	17.5±0.84 ab	17.5±0.54 ab	18.2±0.74 a	18.4±0.15 a	16.9±0.41 bc	16.8±0.54 bc
Food conversion ratio	1.43±0.135 a	1.35±0.044 b	1.36±0.040 b	1.22±0.020 c	1.32±0.015 b	1.32±0.039 b
Protein efficiency ratio	2.49±0.081 c	2.63±0.087 bc	2.62±0.087 bc	2.92±0.082 a	2.70±0.078 b	2.70±0.078 b
Apparent protein utilization (%)	44.42±1.11 c	47.17±1.39 b	47.57±1.12 b	52.64±1.32 a	48.65±1.19 b	48.11±1.10 b
Energy utilization (%)	29.20±0.76 c	30.03±0.67 bc	30.45±0.71 bc	34.02±0.88 a	31.82±1.25 b	31.80±0.77 b

The same letter in the same row is not significantly different at $P < 0.05$.

Table 3. Proximate chemical analysis (%; on dry matter basis) of whole body of Nile tilapia fed practical diets containing different levels of Spirulina for 12 weeks.

Items (%)	Spirulina levels (g/kg diet)					
	Control (0.0)	1.25	2.5	5.0	7.5	10.0
Moisture	72.5±0.27 a	72.9±0.31 a	72.5±0.37 a	72.5±0.56 a	72.3±0.33 a	72.4±0.19 a
Crude protein	62.0±0.31 b	63.7±0.24 a	63.4±0.33 a	63.4±0.46 a	62.4±0.45 b	62.0±0.49 b
Total lipids	25.5±0.18 a	24.3±0.24 a	24.5±0.41 a	25.1±0.54 a	25.3±0.26 a	25.7±0.18 a
Ash	11.8±0.233 a	11.6±0.053 a	11.9±0.089 a	11.3±0.078 a	11.8±0.021 a	11.9±0.302 a

The same letter in the same row is not significantly different at $P < 0.05$.

Table 4. Red blood cell count (RBC), white blood cell count (WBC), lymphocytes, monocytes, and granulocytes of Nile tilapia fed practical diets containing different levels of Spirulina for 12 weeks.

Items	Spirulina levels (g/kg diet)					
	Control (0.0)	1.25	2.5	5.0	7.5	10.0
RBC ($\times 10^6/\mu\text{L}$)	1.87±0.087 b	1.92±0.079 b	2.07±0.127 ab	2.37±0.153 a	2.48±0.158 a	2.54±0.127 a
WBC ($\times 10^5/\mu\text{L}$)	3.13±0.124 b	3.21±0.133 b	3.29±0.176 ab	3.74±0.098 a	3.97±0.127 a	4.02±0.133 a
Lymphocytes (%)	84.4±0.29 c	88.1±2.59 bc	93.2±2.71 ab	94.1±2.71 ab	94.1±2.71 ab	96.4±1.62 a
Monocytes (%)	9.2±0.29 a	8.1±0.29 a	5.0±0.17 b	4.6±0.15 b	4.6±0.15 b	2.8±0.06 c
Granulocytes (%)	6.4±0.23 a	3.8±0.06 b	1.8±0.06 c	1.3±0.06 c	1.3±0.06 c	0.8±0.06 d

The same letter in the same row is not significantly different at $P < 0.05$.

Table 5. Biochemical changes of fry Nile tilapia fed practical diets containing different levels of Spirulina for 12 weeks.

Items (%)	Spirulina levels (g/kg diet)					
	Control (0.0)	1.25	2.5	5.0	7.5	10.0
Glucose (mg/L)	118.4±2.62 d	171.8±3.76 c	183.4±2.53 b	217.3±5.57 a	216.7±7.11 a	221.9±6.04 a
Total lipids (g/L)	31.4±2.92 b	32.5±4.36 b	35.9±2.31 ab	36.1±4.15 ab	39.6±5.22 ab	41.3±2.73 a
Total protein (g/L)	19.6±1.24 c	24.3±2.97 c	25.9±1.28 bc	30.1±4.07 ab	34.7±2.67 a	35.8±3.12 a
Albumin (g/L)	10.1±0.53 c	10.5±0.32 c	12.8±1.61 b	13.9±1.77 b	15.9±2.42 ab	17.2±2.84 a
Globulin (g/L)	6.3±1.16 d	10.0±1.88 c	14.1±2.89 bc	16.3±2.78 ab	18.8±2.36 a	18.6±3.28 a

The same letter in the same row is not significantly different at P < 0.05.

Table 6. Blood leucocytes oxidative radical production (NBT) and the counts of *A. hydrophila* 24 h-after incubation with serum of Nile tilapia fed different levels of Spirulina for 12 weeks.

Items (%)	Spirulina levels (g/kg diet)					
	Control (0.0)	1.25	2.5	5.0	7.5	10.0
NBT (mg/mL)	0.212±0.010 d	0.220±0.012 c	0.277±0.088 b	0.297±0.020 ab	0.217±0.067 a	0.222±0.015 a
Bacterial count (x10 ⁴ cell)	82.7±3.28 a	68.0±1.73 b	61.2±1.73 c	46.7±2.96 d	42.3±2.03 d	43.3±2.03 d

The same letter in the same row is not significantly different at P < 0.05.

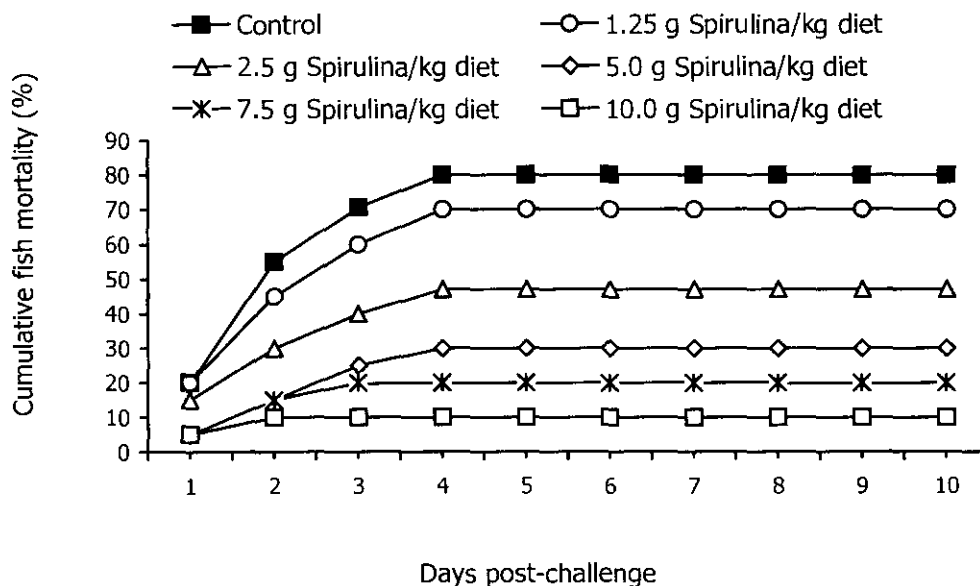


Figure 1. Cumulative mortality rate (No. /day) of Nile tilapia fed a 30%-CP diet with different levels of *Spirulina* in glass aquaria for 12 weeks and challenged by *A. hydrophila* for 10 days

DISCUSSION

Fish fed diets containing *Spirulina* (5.0 - 10.0 g/kg) had significantly better growth and feed utilization as compared to fish fed the control diet. The present study proved that dietary supplementation of *Spirulina* enhanced fish growth and immunity, as brewers yeast, which had been reported to enhance the growth and immunity of Nile tilapia (Lara-Flores *et al.*, 2003; Abdel-Tawwab *et al.*, 2008). These results may possibly due to the improved feed intake and nutrient digestibility. Moreover, *Spirulina* contains several nutrients especially vitamins and minerals that may help in fish growth promotion. These results agree with those found by Belay *et al.* (1996), Hayashi *et al.* (1998), Hirahashi *et al.* (2002) who reported that feeding *Spirulina* to fish and poultry improved survival and growth rates. In this regard, Watanabe *et al.* (1990) mentioned that feed supplemented with *Spirulina* powder improved the feed conversion ratio and growth rates for striped jack, *Pseudocaranx dentex*. Similar results were obtained when yeasts were added to fish diet (Tovar *et al.*, 2002; Lara-Flores *et al.*, 2003; Waché *et al.*, 2006; Abdel-Tawwab *et al.*, 2008).

The *Spirulina* supplementation significantly affected the whole-fish body composition. The better feed intake in *Spirulina* supplemented diets may have been due to the increased fish appetite resulting in a higher feed intake and therefore

improved growth. On the other hand, changes in protein and lipids contents in fish body could be linked with changes in their synthesis, deposition rate in muscle and/or different growth rate (Smith, 1981; Fauconneau, 1984; Soivio *et al.*, 1989; Abdel-Tawwab *et al.*, 2006).

In the present study, fish fed on diets containing 5.0 - 10.0 g Spirulina/kg diet exhibited higher RBCs and WBCs counts, glucose, lipids, protein, albumin, and globulin as compared with fish fed the control diet. These results proved the improvement of fish health when fed Spirulina-supplemented diets. Moreover, the measurement of albumin, globulin, and total protein in serum or plasma is of considerable diagnostic value in fish, as it affects the general nutritional status as well as the integrity of the vascular system and liver function (Schaperclaus *et al.*, 1992).

In the present study, the results of bacteria challenge, bacteriocidal activity, and NBT suggest the increase in phagocytosis in blood, which have an important role for prevention of infectious disease. Phagocytosis by these cells is a process of internalization, killing and digestion of invading microorganisms. In phagocytosis, phagocytes produce oxygen free radicals during the respiratory burst, which is toxic to bacteria. Several authors reported that phagocytosis is stimulated by oral administration of probiotics (Rengpipat *et al.*, 2000; Li and Gatlin, 2004; Panigrahi *et al.*, 2005; Taoka *et al.*, 2006).

In this study, Spirulina could stimulate the immune system via increasing the phagocytic and the natural killer activities (Qureshi and Ali, 1996). This finding agrees with Duncan and Klesius (1996) who reported that Spirulina are capable of enhancing non-specific immune responses in fish. However, they demonstrated that peritoneal phagocytes from channel catfish, *I. punctatus* fed *Spirulina plantesis*, showed enhanced phagocytosis to zymosan and increased chemotaxis to *Edwardsiella ictaluri* exoantigen. Also, Watanuki *et al.* (2006) reported that Spirulina activated the functions of leucocytes, such as phagocytosis and production of superoxide, and cytokines production in common carp, *Cyprinus carpio*.

It is very important to estimate the useful impacts on fish treated with immuno-stimulants. In this study, the obtained results showed that tilapia fed 5.0 - 10.0 g Spirulina/kg diet increased the fish resistance against *A. hydrophyla*. In this regard, Watanuki *et al.* (2006) estimated the fluctuation in the number of bacterial cells in Spirulina-treated fish organs after an artificial challenge with *A. hydrophila*. They found that the bacteria numbers were lower in the liver and kidney of carp treated with Spirulina than the control group suggesting the increased resistance *A. hydrophila* infection.

Spirulina contains phytopigments such as phycobilins, phycocyanin and allophycocyanin, and xanthophylls, which seem to be related to its antioxidant activity (Miranda *et al.*, 1998; Bhat and Madyastha, 2000; Wang *et al.*, 2007; Bermejo *et al.*, 2008). Moreover, Abd El-Baky *et al.* (2004), Wu *et al.* (2005), and Hu *et al.* (2008) reported that the algal carotenoid extract had significantly antioxidant activity. The usefulness of antioxidants is to protect cellular components against oxidative stress (Mohan *et al.*, 2006).

The present study concluded that Spirulina positively improved growth performance and feed efficiency of Nile tilapia as well as its resistance to *A. hydrophila* infection. In addition, this study found that the optimum rate of Spirulina in the fish practical diet is 5.0 – 10.0 g/kg.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Mustafa M. Samy, Botany Department, Faculty of Science, Zagazig University, for providing the authors with Spirulina biomass.

REFERENCES

1. Abd El-Baky, H.H., F.K. El-Baz and G.S. El-Baroty. 2004. Production of antioxidant by green alga *Dunaliella salina*. Intern. J. Agricult. Biol., 6: 49–57.
2. Abdelghany, E. A. 1993. Food and feeding habits of Nile tilapia from the Nile River at Cairo, Egypt. Proceeding of the First International Conference on Fish Farm Technology, Trondheim, Norway, 9-12 August 1993.
3. Abdel-Tawwab, M. and H. I. El-Marakby. 2004. Length-weight relationship, natural food and feeding selectivity of Nile tilapia; *Oreochromis niloticus* (L.) in fertilized earthen ponds. In: R. Bolivar, G. Mair and K. Fitzsimmons (eds.), The 6th International Symposium of Tilapia in Aquaculture ISTA 6, 14-16 September 2004, Manila, Philippines, pp 500-509.
4. Abdel-Tawwab, M., A. M. Abdel-Rahman and N. E. M. Ismael. 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.
5. Abdel-Tawwab, M., Y. A. E. Khatib, M. H. Ahmad and A. M. E. Shalaby. 2006. Compensatory growth, feed utilization, whole body composition and hematological changes in starved juvenile Nile tilapia, *Oreochromis niloticus* (L.). Journal of Applied Aquaculture, 18(3): 17-36.

6. AOAC (Association of Official Analytical Chemists) 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, 15th ed. Association of Official Analytical chemists, Arlington, VA.
7. Belay, A., T. Kato and Y. Ota. 1996. *Spirulina (Arthrospira)*: potential application as an animal feed supplement. International Association of Applied Algology 7th International Conference Abstracts, p. 23.
8. Bermejo, P., E. Piñero and A. M^aVillar. 2008. Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of *Spirulina platensis*. Food Chemistry, 110: 436–445.
9. Bhat, V. B. and K. M. Madyastha. 2000. C-phycocyanin: A potent peroxy radical scavenger in vivo and in vitro. Bioch. Biophys. Res. Comm., 275: 20–25.
10. Boyd, C. E. 1984. Water Quality in Warm water Fishponds. Auburn University Agriculture Experimental Station, Auburn, AL, USA.
11. Brown, B. A. 1988. Routine hematology procedures. Pages 7–122 in B. A Brown, editor. Hematology, principles and procedures. Leo and Fabiger, Philadelphia, Pennsylvania, USA.
12. Cohen, Z. and A. Vonshak. 1991. Fatty acid composition of Spirulina and Spirulina-like cyanobacteria in relation to their chemotaxonomy. Phytochemistry, 30: 205–206.
13. Duncan, P. L. and P. H. Klesius. 1996. Effects of feeding Spirulina on specific and non-specific immune responses of channel catfish. J. Aquat. Animal Health, 8: 308–313.
14. Dytham, C., 1999. Choosing and Using Statistics: A Biologist's Guide. Blackwell Science Ltd., London, UK.
15. El-Sayed, A.-F. M. 2006. Tilapia Culture. CABI publishing, CABI International Willingford, Oxfordshire, UK.
16. Fauconneau, B., 1984. The measurements of whole body protein synthesis in larval and juvenile carp (*Cyprinus carpio* L.). Comp. Biochem. Physiol., 78: 845–850.
17. Gatlin, D. M. III. 2002. Nutrition and fish health. In: Halver, J. E., Hardy, R. W. (Eds). Fish Nutrition. Academic Press, San Diego, CA, USA, pp 671 - 702.
18. Getabu, A., 1994. A comparative study on the feeding habits of *Oreochromis niloticus* (Linnaeus) in Nyanza Gulf Lake Victoria and sewage fish ponds. In: Okemwa, E., Wakwabi, E.O., Getabu, A. (Eds.) Proceedings of the Second EEC

- Regional Seminar on Recent Trends of Research on Lake Victoria Fisheries, Nairobi : ICIPE Science, pp 93-103.
19. Hayashi, O., T. Hirahashi, T. Katoh, H. Miyajima, T. Hirano and Y. Okuwaki. 1998. Class specific influence of dietary *Spirulina plantesis* on antibody production in mice. *J. Nutr. Sci. Vitaminol.*, 44: 841–851.
 20. Henry, R. J. 1964. Colorimetric determination of total protein. In: *Clinical Chemistry*. Harper and Row Publ., New York, USA, p 181.
 21. Hirahashi, T., M. Matsumoto, K. Hazeki, Y. Saeki, M. Ui and T. Seya. 2002. Activation of the human innate immune system by *Spirulina*: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of *Spirulina plantesis*. *Int. Immunopharmacol.*, 2: 423–434.
 22. Hu, C.-C., J.-T. Lin, F.-J. Lu, F.-P. Chou and D.-J. Yang. 2008. Determination of carotenoids in *Dunaliella salina* cultivated in Taiwan and antioxidant capacity of the algal carotenoid extract. *Food Chem.*, 109: 439–446.
 23. Joseph, A., M. Knight, S. Anderson, M. James and H. Rawie. 1972. Chemical basis of the sulfophospho-vanillin reaction for estimating total serum lipid. *Cl. Chem.*, 18(3); 198-201.
 24. Lara-Flores, M., M. A. Olvera-Novoa, B. E. Guzmán-Méndez and W. López-Madrid. 2003. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 216: 193–201.
 25. Li, P. and D. M. Gatlin. 2004. Dietary brewers yeast and the prebiotic GroBiotick™ AE influence growth performance, immune responses and resistance of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) to *Streptococcus iniae* infection. *Aquaculture*, 231: 445– 456.
 26. Lin, W., B. Pan, J. Sheng, J. Xu and Q. Hu. 2007. Antioxidant activity of *Spirulina platensis* extracts by supercritical carbon dioxide extraction. *Food Chem.*, 105: 36–41.
 27. Lu, J. and T. Takeuchi. 2004. Spawning and egg quality of tilapia *Oreochromis niloticus* fed solely on raw *Spirulina* throughout three generations. *Aquaculture*, 234: 625-640.
 28. Lu, J., G. Yoshizaki, K. Sakai and T. Takeuchi. 2002. Acceptability of raw *Spirulina platensis* by larval tilapia *Oreochromis niloticus*. *Fish. Sci.*, 68: 51-58.

29. Madhava, C., V. B. Bhat, G. Kiranmai, M. N. Reddy, P. Reddanna and K. M. Madyastha. 2000. Selective inhibition of cyclooxygenase-2 by C-phycoyanin, a biliprotein from *Spirulina platensis*. *Bioch. Biophys. Res. Comm.*, 277: 599–603.
30. Mahajan, G. and M. Kamat. 1995. c-Linoleic acid production from *Spirulina platensis*. *Appl. Microbiol. Biotech.*, 43: 466–469.
31. Miranda, M. S., R. G. Cintra, S. B., Barros and F. J. Mancini. 1998. Antioxidant activity of the microalga *Spirulina maxima*. *Braz. J. Med. Biol. Res.*, 31(8): 1075–1079.
32. Mohan, I. K., M. Khan, J. C. Shobha, M. UC. Naidu, A. Prayag and P. Kuppusamy. 2006. Protection against cisplatin-induced nephrotoxicity by *Spirulina* in rats. *Cancer Chem. Pharm.*, 58(6): 802–808.
33. Morist, A., J. L. Montesinos, J. A. Cusido and F. Godia. 2001. Recovery and treatment of *Spirulina platensis* cells cultured in a continuous photobioreactor to be used as food. *Process Biochem.*, 37: 535–547.
34. Nandeesh, M. C., B. Gangadhara, T. J. Varghese and P. Keshavanath. 1998. Effect of feeding *Spirulina platensis* on the growth, proximate composition and organoleptic quality of common carp, *Cyprinus carpio*. *Aquacult. Res.*, 29: 305-312.
35. Nandeesh, M. C., B. Gangadhara, J. K. Manissery and L. V. Venkataraman. 2001. Growth performance of two Indian major carps, catla (*Catla catla*) and rohu (*Labeo rohita*) fed diets containing different levels of *Spirulina platensis*. *Bioresource Technology*, 80: 117-120.
36. Okada, Y, N. J. Klein and J. Pierro. 1999. Neutrophil dysfunction: The cellular mechanisms of impaired immunity during total parenteral nutrition in infancy. *J. Pediatr. Surg.*, 34: 242–245.
37. Palmegiano, G. B., E. Agradi, G. Forneris, E. Gai, L. Gasco, E. Rigamonti, B. Sicuro and I. Zoccarato. 2005. Spirulina as a nutrient source in diets for growing sturgeon (*Acipenser baeri*). *Aquacult. Res.*, 16: 188-195.
38. Palmegiano, G. B., F. Gai, F. Dapra, L. Gasco, M. Pazzaglia and P. G. Peiretti. 2008. Effects of Spirulina and plant oil on the growth and lipid traits of white sturgeon (*Acipenser transmontanus*) fingerlings. *Aquacult. Res.*, 39: 587-595.
39. Panigrahi, A., V. Kiron, J. Puangkaew, T. Kobayashi, S. Satoh and H. Sugita. 2005. The viability of probiotic bacteria in rainbow trout *Oncorhynchus mykiss*. *Aquaculture*, 243: 241–254.

40. Qureshi, M. A. and R. A. Ali. 1996. *Spirulina platensis* exposure enhances macrophage phagocytic function in cats. *Immunopharm. Immunotoxicol.*, 18(3): 457–463.
41. Rengpipat, S., S. Rukpratanporn, S. Piyatiratitivorakul and P. Menasveta. 2000. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus S11*). *Aquaculture*, 191: 271–288.
42. Rook, G. A. W., J. Steele, S. Umar and H. M. Dockrell. 1985. A simple method for the solubilisation of reduced NBT, and its use as a colorimetric assay for activation of human macrophages by γ -interferon. *J. Immunol. Methods*, 82: 161–167.
43. Schaperclaus, W., H. Kulow and K. Schreckenbach. 1992. *Fish Disease*. A.A. Balkema, Rotterdam, the Netherlands.
44. Smith, M. A. K. 1981. Estimation of growth potential by measurement of tissue protein synthetic rates in feeding and fasting rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol.*, 19: 213-220.
45. Soivio, A., M. Niemisto and M. Backstrom. 1989. Fatty acid composition of *Coregonus muksu* tured striped jack. *J. Tokyo Univer. Fish*, 77: 231-239.
46. Watanuki, H., K. Ota, A. C. Malin, A. R. Tassakka, T. Kato and M. Sakai. 2006. Immunostimulant effects of dietary *Spirulina platensis* on carp, *Cyprinus carpio*. *Aquaculture*, 258: 157–163.
47. Wotton, I. D. and H. Freeman. 1982. *Microanalysis in Medical Biochemistry*. Churchill, New York, USA.
48. Wu, L.C., J.A. Ho, M.C. Shieh and I.W. Lu. 2005. Antioxidant and antiproliferative activities of *Spirulina* and *Chlorella* water extracts. *J. Agricult. Food Chem.*, 53(10): 4207–4212.
49. Zarrouk, C., 1966. Contribution à l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima* Geilster. PhD thesis, Université de Paris, Paris, France.