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The Role of *Spirulina platensis* (Arthrospira platensis) in Growth and Immunity of Nile Tilapia (*Oreochromis niloticus*) and Its Resistance to Bacterial Infection

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Abstract

The current study was designed to optimize the dietary levels of *Spirulina platensis* in *Oreochromis niloticus;* this was tested via graded levels. Six isonitrogenous and isocaloric rations containing graded levels of dried spirulina 0, 5, 7.5, 10, 15 and 20 g/kg diet were fed separately to six equal groups of *O. niloticus* fingerlings for 3 months. Growth performance, non-specific immune parameters, tissue reactions and resistance of tilapias post challenge infection with *Pseudomonas fluorescens* were estimated monthly. There were significant increase in growth performance parameters and survival rates in spirulina-supplemented groups at concentration level of 10 g/kg for 2 months. Significant increases in hematocrit, nitroblue tetrazolium and lysozyme activity were observed in most of the supplemented groups. Bacterial challenge infections resulted in significantly lower mortality rate in all Spirulina groups with remarkable increase in protection of fish received 10 g/kg. In sum, it advisable to incorporate 10 g/kg diet of spirulina for 2 months for maximum growth performance, immunity and disease resistance in *O. niloticus*.

Keywords: *Spirulina platensis*, growth performance, hematocrit, nitroblue tetrazolium, lysozyme activity, challenge infection

1. Introduction

The increase in aquaculture production could be accomplished either by increasing the cultured area or intensification of production. Under such extreme conditions; several growth and immune suppressors can take place (Yousefian & Amiri, 2009), in addition, aquatic organisms are in constant contact with a plethora of bacteria, many of which are pathogenic (Kaizu et al., 2011).

Several sectors of the aquaculture industry would benefit if cultured organisms were conferred with enhanced feed efficiency, growth performance, and disease resistance without environmental conflicts (Gatlin et al., 2006). Functional feed additives strategy has recently gained considerable attention. From nutritional point of view; it does not only provide the essential nutrients required for normal physiological functioning, but also serve as a medium by which fish receive other components that may positively affect their health (Ibrahem et al., 2010) . Researches on diets optimization to enhance fish health and production are still in infancy.

There are increasing recognition for the importance of aquatic macrophytes as feed in aquaculture. Tilapias are omnivorous that can utilize cyanobacterial blue-green algae (Chow & Woo, 1990). *Spirulina platensis* (SP), a filamentous cyanobacterium, possesses diverse biological and nutritional significance. It has the potentiality to produce large numbers of antimicrobial substances; therefore, it is considered a suitable candidate for exploitation as bio-control agent against pathogenic micro-organisms (Ozdemir et al., 2004). In aquaculture, different forms of SP were tested in various fish and shellfish species (Ungsethaphand et al., 2010).

Optimization of dried form of whole *S. platensis* concentration in fish diets is still questionable, regarding its optimum concentration needed to reach its desired effects on growth performance, feed utilization, immune responses, and resistance of tilapias to infections.

SP is well known for its anti-oxidant and anti-cancerous properties. A hot water extract of SP has been orally administered to patients and proved as an anti-cancer agent. SP hinders the growth of oral cancer in Syrian hamster cheek (Grawish, 2008). Such an inhibitory effect may be attributed to the repair of carcinogen- damaged DNA, meanwhile, SP has been suggested as an efficient radical scavenger (Romay et al., 1998). Several studies reported the unique role of SP polysaccharides in enhancing the cell nucleus enzyme activity and the process of DNA repair (Kaji et al., 2002).

Therefore, the overall goals of the present study are to investigate the optimum dietary concentration and duration of dried *S. platensis* needed to exert its potential effects on growth performance, immunomodulator, chemoprotective agent, in addition to the resistance of *O. niloticus* supplemented groups to *Pseudomonas fluorescens* infections.

2. Materials and Methods

2.1 Fish

A total of 2400 *O. niloticus* fries (mean individual initial weight 4 ± 1.0 g) were obtained from Abbasa hatchery, they were divided into six equal groups, each consisting of four replicates (100 fry/replicate) in 6 separate earthen ponds, Fish in each replicate were reared in a hapa made of cotton mesh like a cage (3 × 2 × 1 m, each) that was fixed in an earthen pond (for each group, a total of 4 hapas were equally arranged in 4rows). The whole experiment was done at the experimental units of The World Fish centre, Abbasa, Sharkia, Egypt. The Fish were fed twice daily on a basal diet of 35% protein at 10% of body weight per day. The feed was placed in plastic trays fitted in the hapas (one per hapa). The water was partially renewed daily and monitored regularly; the water temperature was maintained at 25 ± 1°C.

2.2 Spirulina Platensis

Pure dried S. platensis (*Arthrospira platensis*) tablets were obtained from Lake Heath Products Co., Ltd. Liyang City, Jiangsu Province, China. It was dark pure green in colour with smooth surface. The tablets were grounded to a powder form before usage.

2.3 Rations

A standard commercial ration containing crude protein, crude lipid, vitamins and minerals met the basic dietary requirements of Nile tilapia was prepared (Table 1). The ingredients were mixed mechanically by the horizontal mixer (Hobarts model D300-T, Troy, OH, USA). The pellets were then prepared using a pellet-machine (California Pellet Mill, Roskamp Huller Co.) with 0.5 cm diameter and pellets were left for 24 h for air-drying at room temperature (28°C), broken into small pieces and sieved to obtain the appropriate size. The rations were transferred into plastic bags and stored in a refrigerator at 4°C until used. Six experimental rations were prepared. The first five groups were prepared by mixing separately a graded concentration of *S. platensis 5*, 7.5, 10, 15 and 20 g *S. platensis* /kg diet. The last group was assigned as control ration which consisted of the standard commercial ration without any treatment. The required diet was prepared biweekly and stored in a refrigerator (4°C) for daily use.

Table 1. Composition of the Oreochromis niloticus basal diet used throughout the experiment

Ingredients	Diet (%)	Protein (%)		Metabolic energy (Joules)	
		ingredients	Feed	Ingredients	feed
Fish meal	7.95	0.72	5.76	4000	32000
Soybean meal	52.8	0.48	25.39	2870	151823
Ground corn	29.1	0.10	3.17	1240	36084
Wheat flour	5.00	0.13	0.67	2700	13500
Vegetable oil	2.00	0.00	0.00	9100	18200
Cod liver oil	2.00	0.00	0.00	9100	18200
Di calcium phosphate	1.00	0.00	0.00	0.00	0000
Mineral mix.	0.07	0.00	0.00	0.00	0000
Vitamin mix.	0.05	0.00	0.00	0.00	0000
Total	100	0.00	34.99	0.00	269807

2.4 Pathogen

Pseudomonas fluorescens was previously isolated from naturally infected *O. niloticus* and identified according to the standard bacteriological tests. The pathogen was cultured in Tryptic Soya broth (TSB) (Oxoid) for 24 h at 37°C. The broth culture was centrifuged for 10 min at 3000 rpm. The supernatant was discarded and the pellets were re-suspended in phosphate buffered saline at pH 7.4 (PBS 7.4) and the optical density (OD) of the solution was adjusted to 0.5 at 456 nm, which correspond to 1×10^8 cells mL^{-1}. This bacterial suspension was serially diluted using standard dilution technique with PBS 7.4 and used for the challenge experiment.

2.5 Experimental Design

To evaluate the efficacy of SP *on* cultured *O. niloticus*; three month feeding study periods were conducted. The pre-acclimated fish were divided into 6 equal groups. Group 1 was fed on a basal diet (control) and the five groups were dietary supplemented with single graded concentration of dried SP 5, 7.5, 10, 15 and 20 g kg^{-1} diet fed, respectively. Groups were evaluated for growth performance expressed as survival, specific growth rate, and condition factor. Blood samples were collected after 1, 2, and 3 months for hematological analysis and immunological investigations. At the end of each month, the mortalities as well as the relative level of protection were estimated post challenge infections using *P. fluorescens*.

2.6 Growth Performance and Survival

Fish of all replicates were weighed individually and their body weight gain was measured. Specific growth rate (SGR) and condition factor (CF) were calculated according to Goodwin et al. (1983). The survival percentage was recorded along the period of experiment.

$$SGR = \text{Ln [final mean body weight (g)]-Ln [initial mean body weight (g)]} \div \text{time interval (days)} \times 100$$

$$CF = \text{weight (g)} \div [\text{length (cm)}]^3$$

3. Hematological and Immunological Analysis

3.1 Blood Sampling

Twenty fish were randomly collected from each group and were anesthetized via immersing in water containing tricaine methane sulfonate (MS-222) neutralized by sodium bicarbonate. Whole blood (0.5 ml) pooled samples were collected from the caudal vessels of each fish using syringes (1 ml) and 27-gauge needles rinsed with heparin (15 unit/m).

A further 0.5 ml blood sample was centrifuged at 2000 rpm for 5 min in order to separate the plasma. The latter was stored at -20°C.

3.2 Hematocrit (HCT) Values

Hematocrit capillary tubes were two-third filled with the whole blood and centrifuged in a hematocrit centrifuge for 5 min and the percentage of the packed cell-volume was determined by the hematocrit tube reader.

3.3 Nitroblue Tetrazolium (NBT) Activity

The production of oxygen radicals from phagocytes in the blood was measured using nitroblue tetrazolium (NBT) dye as described by Anderson and Siwicki (2005). Briefly, blood (0.1 ml) was placed in microtiter plate wells, to which an equal amount of 0.2% NBT solution was added and incubated for 30 min at room temperature. A sample of NBT blood cell suspension (0.05 ml) was added to a glass tube containing 1 ml N,N-dimethyl formamide and centrifuged for 5 min at 3000 rpm. The supernatant fluid was measured in a spectrophotometer at 620 nm in 1 ml cuvettes.

3.4 Adherence/NBT Assays

Nitroblue tetrazolium-glass adherent assays (NBT-glass adherent assay) were performed by placing single drops of blood (0.1 ml) on 2 glass cover slips and incubating them for 30 min at room temperature. The cover slips were then gently washed with phosphate buffered saline (PBS). Drops (0.1 ml) of 0.2% NBT were placed on microscope slides and covered by a cover slip, then incubated at room temperature for 30 min with the NBT solution. The activated neutrophils were then counted under electric light microscope (×400).

3.5 Lysozyme Activity

The lysozyme activity was measured using the turbidity assay. Chicken egg lysozyme (Sigma) was used as a standard and 0.2 mg/ml lyophilized *Micrococcus lysodeikticus* in 0.04 M sodium phosphate buffer (pH 5.75) was used as a substrate. Plasma (50 μg) samples were added to 2 ml of bacterial suspension and the reduction in the

absorbance at 540 nm was determined after 0.5 and 4.5 min incubation at 22ºC. One unit of lysozyme activity was defined as a reduction in absorbance of 0.001 min^{-1}.

3.6 Challenge of Fish

For the challenge experiments; The supplemented and the control groups were subjected to three challenge infections, post 1, 2 and 3 months feeding trials; for this purpose; a total of 72 fish from each treatment (18 from each replicates) were used for challenge test, these fish were divided into two groups (each 36). Each group was subdivided into 3 subgroups (replicates, each 12) each subgroup reared in glass aquaria (50 × 60 × 70 cm). The groups were injected intraperitoneally with 0.5 ml of 4×10^8 *P. fluorescens*. The challenged fish, from each aquarium, were observed for 10 days in order to record the daily mortality.

The relative level of protection (RLP) among the challenged fish was determined

RLP % = 1 – (percent of mortality in treated groups/ percent of mortality in control group) × 100.

4. Statistical Analysis

The mean and standard error were calculated for each variable. The data were analyzed by analysis of variance (ANOVA) to identify the significantly different groups at ($P < 0.05$) by one way ANOVA with post hoc LSD multiple comparison test using SPSS software statistical program (SPSS for windows ver.15.00, USA).

5. Results

5.1 Growth Performance

Our study revealed that the growth performance expressed as body weight gain, specific growth rates, and condition factor, were significant ($p \leq 0.05$) in groups 3 & 4 in all periods versus the control group, with higher mean value level is in group 4 (Table 2).

Table 2. Growth performance and survival of experimental tilapia at end of the second month of feeding supplemented diet with Spirulina*, **

Month	Group	Spirulina Dose	Parameter							
			Body Gain (g)		Specific Growth Rate %		Condition Factor %		Survival %	
			Main	± SE	Main	± SE	Main	± SE	Main	± SE
First month	Gp. 1	Control (Zero Dose)	8.48	0.68 ^{C}	1.88	0.10 ^{C}	1.67	0.02 ^{C}	87.67	1.45 ^{A}
	Gp. 2	5g/kg	12.56	0.78 ^{B}	2.29	0.11 ^{BC}	1.82	0.02 ^{B}	88.67	2.40 ^{A}
	Gp. 3	7.5g/kg	18.79	1.28 ^{A}	2.39	0.13 ^{AB}	1.82	0.02 ^{AB}	91.00	2.08 ^{A}
	Gp. 4	10g/kg	20.33	0.99 ^{A}	2.63	0.09 ^{A}	1.88	0.02 ^{A}	91.67	2.03 ^{A}
	Gp. 5	15 g/kg	21.05	1.11 ^{A}	2.72	0.12 ^{A}	1.94	0.03 ^{A}	92.99	3.04 ^{A}
	Gp. 6	20 g/kg	21.95	0.95 ^{A}	2.84	0.08 ^{A}	1.97	0.02 ^{A}	94.00	3.33 ^{A}
Second month	Gp .1	Control (Zero Dose)	19.04	1.24 ^{C}	1.51	0.06 ^{C}	1.82	0.02 ^{C}	81.67	1.67 ^{B}
	Gp. 2	5g/kg	19.50	1.39 ^{B}	1.88	0.06 ^{B}	1.94	0.02 ^{B}	83.00	1.53 ^{AB}
	Gp. 3	7.5g/kg	31.10	2.00 ^{B}	2.04	0.07 ^{B}	1.99	0.02 ^{B}	85.67	1.20 ^{AB}
	Gp. 4	10g/kg	37.39	2.16 ^{A}	2.27	0.07 ^{A}	2.21	0.03 ^{A}	87.67	1.45 ^{A}
	Gp. 5	15 g/kg	39.61	2.11 ^{A}	2.40	0.06 ^{A}	2.24	0.04 ^{A}	91.00	2.04 ^{A}
	Gp. 6	20 g/kg	42.33	1.99 ^{A}	2.57	0.08 ^{A}	2.29	0.02 ^{A}	92.20	1.98 ^{A}
Third month	Gp. 1	Control (Zero Dose)	35.33	2.29 ^{C}	1.46	0.05 ^{C}	1.79	0.02 ^{B}	79.00	2.51 ^{B}
	Gp. 2	5g/kg	43.33	2.89 ^{Bc}	1.60	0.06 ^{Bc}	1.84	0.03 ^{B}	81.33 ^{B}	1.33 ^{A}
	Gp. 3	7.5g/kg	51.75	4.12 ^{AB}	1.74	0.06 ^{AB}	2.02	0.03 ^{A}	83.67 ^{B}	0.88 ^{A}
	Gp. 4	10g/kg	57.68	4.31 ^{A}	1.83	0.07 ^{A}	2.10	0.03 ^{A}	85.67	1.20 ^{A}
	Gp. 5	15 g/kg	59.90	5.11 ^{A}	1.85	0.06 ^{A}	2.20	0.03 ^{A}	88.45	1.11 ^{A}
	Gp. 6	20 g/kg	62.34	3.88 ^{A}	1.93	0.08 ^{A}	2.29	0.05 ^{A}	89.10	0.98 ^{A}

5.2 The Survival Rate

During the feeding experiment, the survival % expressed as mean ±SE showed significant increases in group 4 at 2 month of experiment and in all *S. platensis* supplemented groups vs. the control group after the third month of experiment (Table 2).

5.3 Hematological and Immunological Analysis

A significant ($p \leq 0.05$) increase of hematocrit (HCT) and nitroblue tetrazolium (NBT) were observed in all (with the exception of group 2 in case of NBT at 1st and 2nd month) *S. platensis* supplemented groups vs. the control group at all experimental period. On the other hand, Neutrophil Adherence values showed significant ($p \leq 0.05$) increase in all *S. platensis* supplemented groups vs. the control group (Table 3). The increase in the lysozyme activity was significant in all fish groups given basal diet supplemented with *S. platensis* at the 2nd and 3rd month (Table 2).

Table 3. Some hematological and immunological parameters of experimental tilapia at end of the first month of feeding supplemented diet with Spirulina*, **

Month	Group	Spirulina Dose	Parameter							
			HCT		NBT		Nutrophyl Adherence		Lysozyme activity	
			Main	± SE	Main	± SE	Main	± SE	Main	± SE
First month	Gp. 1	Control (Zero Dose)	27.25	1.24 ^{B}	0.23	0.02 ^{B}	9.00	1.61^{C}	8.59	1.39 ^{C}
	Gp. 2	5g/kg	30.36	1.89 ^{A}	0.29	0.02 ^{AB}	10.30	1.59^{B}	9.89	1.40 ^{BC}
	Gp. 3	7.5g/kg	31.40	1.00 ^{A}	0.33	0.03 ^{A}	10.99	1.58^{A}	11.25	1.61 ^{AB}
	Gp. 4	10g/kg	32.95	1.10 ^{A}	0.38	0.05 ^{A}	11.80	1.59^{A}	11.65	1.71 ^{A}
	Gp. 5	15 g/kg	33.00	1.3 ^{A}	0.39	0.04^{A}	12.00	1.55^{A}	11.78	1.65^{A}
	Gp. 6	20 g/kg	33.10	1.1 ^{A}	0.41	0.03^{A}	12.20	1.48^{A}	11.90	1.72^{A}
Second month	Gp .1	Control (Zero Dose)	28.42	1.58 ^{B}	0.27	0.02 ^{C}	9.56	1.37^{B}	8.97	1.39^{C}
	Gp. 2	5g/kg	31.38	1.02^{A}	0.34	0.03 ^{A}	11.30	1.52^{A}	10.26	1.45^{B}
	Gp. 3	7.5g/kg	31.90	1.23^{A}	0.40	0.03 ^{AB}	11.41	1.41^{A}	11.80	1.44^{A}
	Gp. 4	10g/kg	33.20	1.00^{A}	0.49	0.02 ^{A}	12.21	1.26^{A}	12.20	1.33^{A}
	Gp. 5	15 g/kg	33.50	1.44^{A}	0.51	0.04^{A}	12.54	1.37^{A}	12.58	1.44^{A}
	Gp. 6	20 g/kg	33.75	1.32^{A}	0.52	0.03^{A}	12.65	1.44^{A}	12.67	1.24^{A}
Third month	Gp. 1	Control (Zero Dose)	28.80	1.65^{B}	0.29	0.02^{B}	9.80	1.29^{B}	9.00	1.33^{C}
	Gp. 2	5g/kg	33.30	1.49^{A}	0.35	0.01^{AB}	10.40	1.31^{B}	10.10	1.31^{B}
	Gp. 3	7.5g/kg	33.40	1.52^{A}	0.36	0.02^{A}	11.50	1.23^{A}	10.40	1.27^{B}
	Gp. 4	10g/kg	33.80	1.47^{A}	0.38	0.03^{A}	11.70	1.26^{A}	11.90	1.43^{A}
	Gp. 5	15 g/kg	34.10	1.48^{A}	0.39	0.02^{A}	11.80	1.32^{A}	12.00	1.33^{A}
	Gp. 6	20 g/kg	34.44	1.51^{A}	0.35	0.04^{A}	12.00	1.25^{A}	12.22	1.28^{A}

5.4 The Mortality Rate

Following the challenge infection using *P. fluorescens* the mortality rate was significantly lower in all *S. platensis* supplemented groups vs. the control throughout the experimental period.

5.5 The Relative Level of Protection (RLP)

The results recorded in (Table 4) evoked significant protection in all *S. platensis* supplemented groups vs. the control.

It worth mentioned that the mean value level is in-group 4 (group received 10 g/kg) is higher in all the tested parameters with significant difference.

Table 4. Mortality and relative level of protection of experimental tilapia at end of the first, second and third months of feeding supplemented diet with Spirulina after challenged with *Pseudomonas florescence**, **

Month	Group	Spirulina Dose	*Pseudomonas florescence*		
			Mortality %		RLP %
			Main	± SE	
First month	Gp.1	Control (Zero Dose)	65.00	5.00 ^{C}	0.00
	Gp.2	5g/kg	60.00	2.89 ^{B}	8.3
	Gp.3	7.5g/kg	58.33	1.67 ^{B}	10.26
	Gp.4	10g/kg	54.67	1.67 ^{AA}	15.8
	Gp. 5	15 g/kg	52.20	1.54 ^{A}	19.69
	Gp. 6	20 g/kg	50.12	2.45 ^{A}	22.89
Second month	Gp.1	Control (Zero Dose)	66.67	1.67 ^{C}	0.00
	Gp.2	5g/kg	56.67	1.67 ^{B}	15.00
	Gp.3	7.5g/kg	53.33	1.67 ^{B}	20.01
	Gp.4	10g/kg	51.67	1.67 ^{A}	22.50
	Gp. 5	15 g/kg	49.45	1.54 ^{A}	25.83
	Gp. 6	20 g/kg	48.00	1.76 ^{A}	28.00
Third month	Gp.1	Control (Zero Dose)	63.33	3.33 ^{C}	0.00
	Gp.2	5g/kg	51.37	1.67 ^{B}	18.60
	Gp.3	7.5g/kg	48.33	1.67 ^{B}	23.70
	Gp.4	10g/kg	41.67	1.33 ^{A}	34.20
	Gp. 5	15 g/kg	40.00	1.42 ^{A}	36.84
	Gp. 6	20 g/kg	38.00	1.54 ^{A}	40.00

***** In all tables: Gp.1: First group, Gp.2: Second group, Gp.3: Third group, Gp.4: Fourth group.

******Means with the same letter in the same column are not significantly different.

6. Discussion

The primary objective in fish nutrition is to provide a nutritionally balanced mixture of ingredients to support the fish vital functions in an acceptable cost (NRC, 1993). *S. platensis* was reported to improve feed efficiency, carcass quality, and physiological response to stress in several species of fish (Takeuchi et al., 2002). None of the previous studies recommended an optimum concentration of dietary *S. platensis* b] ased on graded level study.

Spirulina appeared to be a useful tool to include in the arsenal of disease control and prevention. However, it must not replace good management techniques. The strength of spirulina appears to lie in its ability to improve growth, survival and non-specific immune function against fish pathogens as well as its chemo-protective efficiency. The algae may significantly aid the aquaculture industry.

In the current study we expanded the *S. platensis* dietary concentration to be 0.05, 7.5, 10, 15 and 20 g/kg in order to assess the optimum dietary concentration for *O. niloticus* using pure whole *S. platensis* in powder form.

Specific growth rate (SGR) and condition factor (CF) are the measuring tools reflecting the fish health status under natural and experimental conditions. In the current study pure dried *S. platensis* found to be of potential effects on growth at an optimum concentration of 7.5 and 10 g/kg. It worth mentioned that the mean value level in the group received 10 g/kg is higher in all the tested parameters with significant difference; these results cleared that the optimum dietary level of S. platensis for O. niloticus is 10 g/kg for 2 months to enhance growth performance. Duncan and Klesius (1996) reported that *Spirulina* alga was a good source of protein for animal feed, being containing high amounts of vitamins and minerals, in addition, Nakono et al. (2003) recorded that the lack of cellulose from the cellular structure of *Spirulina* render it easily digestible, thus, increase fish appetite, improve

feed intake and nutrient digestibility and in turn enhance the health of fish, increasing the ability to fight off infections through the reduction of stress levels. The results in the current study are in accordance with Watanabe et al. (1990) and Takeuchi et al. (2002) who found that feed supplemented with S. platensis powder improved the feed conversion ratio and growth rates in striped jack, Pseudocaranx dentex. Lu et al. (2002) demonstrated that raw S. platensis can be an effective uni-feed for larval tilapia at a feeding rate of 30% (on a dry basis) of body weight. Abdel-Tawwab and Ahmed (2009) recorded that the growth and feed utilization of O. niloticus were obtained at 5.0 g fresh culture of S. platensis /kg diet. On the contrary, Ungsethaphand et al. (2010) recorded that the final weight gain, specific growth rate, feed conversion ratio of hybrid red tilapia were not affected by *S. platensis* supplementation. These variations might be attributed to the difference in the *S. platensis* concentration to exert the intended effects, the form of *S. platensis*, raw or dried *S. platensis* or even its products, fish species and size in addition to the rearing conditions.

The high survival rate results in the current study are consistent with that recorded by Dernekbasi et al. (1993) who observed good survival rates in all Guppies treated with *S. platensis* using 40% *S. platensis* supplementation. However, the previous study did not mention the form of *S. platensis* used; in addition, the difference in the *spirulina* concentration could be related to the difference in the feeding habits of the fish species. In contrary to our results Abdel-Tawwab and Ahmed (2009), and Ungsethaphand et al. (2010) found no significant changes in the survival rates of fish dietary supplemented with *S. platensis* at different concentrations.

Adherence/NBT (nitroblue tetrazolium) and respiratory burst process assay spotlights on the non-specific immune response and the antibacterial mechanisms of the tested substances. The results of the current feeding trial showed a significant increase of nitroblue tetrazolium (NBT) and Neutrophil Adherence values in most of the *S. platensis* supplemented groups. The current results agree with those of Duncan and Klesius (1996) who reported enhancement of the peritoneal phagocytes from channel catfish, *I. punctatus* fed *S. platensis*, *Spirulina* algae contain carotenoids, which specifically improving fish health and increasing the ability to fight off infections through the reduction of stress levels. In addition, Watanuki et al. (1990) reported that *S. platensis* activated the functions of leucocytes, such as phagocytosis and production of superoxide, and cytokines production in common carp, *Cyprinus carpio*.

The present study found that fish fed with 10% spirulina exhibited significant haematocrit values. The increase in the immunity stimulating capacity (measure by a lysozyme activity assay) was significant in all fish groups given basal diet supplemented with *S. platensis* at the 2nd and 3rd month.

Lysozyme is found in a wide range of vertebrates including fish and is one of the defensive factors against invasion by microorganisms. The increase in the immunity stimulating capacity could be due to the presence of C-phycocyanin in the *Spirulina* alga, which can help build the immunity capacity (Vonshak, 1997). Results were in accordance with Tayag et al. (2010) who concluded that the white shrimp *L. vannamei* that received the hot-water extract of *S. platensis* had enhanced innate immunity as lysozyme and increased resistance against *V. alginolyticus* infection.

The challenge infection revealed a significantly lower mortality percentage in the group received 10 g/kg *S. platensis* in diet for the 1, 2 and 3 months feeding trial and significantly high relative level of protection (RLP) after challenge infection using *P. fluorescens*. The disease challenge is an in vitro technique provides an opportunity to determine the performance and immunity of the fish species ebon exposure to xenobiotic (bacteria) on their natural habitats (AraKoosh et al., 2009). The results were in accordance with Watanuki et al. (2006) who recorded an increased resistance of *S. platensis* treated carp ebon artificial challenge with *A. hydrophila* than the control group. Abdel-Tawwab and Ahmad (2009) found that SP has a useful impact on fish as immuno-stimulants, they recorded that tilapia fed 5-10 g *fresh S. platensis* /kg diet increased its resistance against *A. hydrophyla.*

Finally, from the present investigation, it was concluded that optimized the dietary levels of dried Spirulina in *O. niloticus,* was useful to decrease costs of the products used and negate losses that could be encountered with improper supplementation levels. It was proved that the optimum concentration of dried *S. platensis* in the *O. niloticus* practical diet is 10 g/kg for 2 months, to positively improving health conditions*,* enhanced the non-specific immunity of *Oreochromis niloticus,* as well as its resistance to challenge by *P. fluorescens* infections. It is recommended to supplement Spirulina in the diet of Nile tilapia especially those grow in farms under immunosuppressive/stressful conditions. Additional researches are needed to study additional desired effects of the blue green algae in cultured fish.

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