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EFFECT OF SPIRULINA ENRICHED DIET ON GROWTH AND HEALTH OF *OREOCHROMIS NILOTICUS* AND PROTECTIVE EFFECT AGAINST INFECTION WITH *AEROMONAS HYDROPHILA*

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Abstract

Effect of dietary supplementation with Spirulina platensis on growth performance, innate immune response, and disease resistance in *Oreochromis niloticus* against *Aeromonas hydrophila* infection is reported. Two hundred and forty *O. niloticus* with average body weight 33±1 g were distributed randomly into four triplicate groups. Fish were fed on four different diets, the control diet (without spirulina additive), while the three other supplemented diets contained Spirulina at 0.5, 1 and 1.5 % respectively for two months. The fish fed with 1% Spirulina enriched diet and control group were inoculated with pathogenic strain of *A. hydrophila*.

The results showed that the fish fed Spirulina enriched diet showed significant increase in final body weight, gain percent and specific growth rate. Also, fish fed Spirulina enriched diet showed significant increase in immunoglobulin M and lysozyme. Supplemented diet with Spirulina has protective fish against the infection with A. *hydrophila*. It advisable to incorporate 1% Spirulina enriched diet for maximum growth performance, immunity and protection against the infection with *A.hydrophila* in *O. niloticus.*

Key words: Spirulina, *Oreochromis niloticus*, growth, Immunity, Health status.

INTRODUCTION

Aim of fish nutrition is to feed a nutritionally balanced mixture of ingredients to aid in the fish vital functions in an acceptable cost (NRC, 1993). Egypt is one of the top ten regional and world aquaculture producers in 2010 for freshwater fish species (FAO, 2012). Fish in intensive system is more susceptible to be infected with several pathogens which may cause mortality

and economic losses (Wang *et al*., 2015). *Aeromonas hydrophila* is one of most dangerous pathogen in fish farms, which causes severe mortalities and losses to tilapia production. The infection is often associated with hemorrhagic septicemia (Giri *et al*., 2013).

Antibiotics, vaccines, and chemotherapeutics are used to guard fishes against diseases, but continue using of antibiotics will produce drug-resistant pathogens, environmental hazards, and food safety concerns (Austin and Austin, 2007). Presence of various pathogens in fish farm will hinder the action of vaccines (Harikrishnan *et al*., 2009). Therefore, it is important to use natural therapeutics approach to ensure health and high productivity of aquaculture. The response of fish to infectious agents is by two mechanisms specific and nonspecific, but they depend on non-specific immune responses (Misra *et al*., 2006). Increasing fish immunity is possibly the best in disease prevention. The products of several herbal plants and algae have been used to boost the immunity of fish, including Spirulina, Astragalus radix and Ganoderma lucidum (Yin *et al*., 2009).

Spirulina (Spirulina platensis) is a marine blue-green filamentous alga that grows in carbonate-rich lakes. Cyanobacterium has been produced commercially as a human food supplement as it is rich with high-quality protein and many nutritional components such as vitamins, minerals, essential fatty acids and B-carotene (Hayashi *et al*., 1998). S. platensis was used to enhance feed efficiency, and physiological response to stress in several species of fish (Takeuchi *et al*., 2002). Recently, Spirulina has been speculated to be associated with modulation of the host immune system and suggested that immune-stimulatory effects could be achieved by dietary Spirulina on carp (Hironobu *et al*., 2006). Therefore, the aim of the present study is to detect the optimum level of dried S. platensis needed to exert its potential effects on growth performance, immunomodulatory and disease resistance in *Oreochromis niloticus* against *Aeromonas hydrophila* infections.

MATERIALS AND METHODS

Experimental fish:

Two hundred and forty apparently healthy live *Oreochromis niloticus* with an average body weight 33±1.0 g acquired from Abassa Fish Farm at Sharkia governorate. Fish were kept in glass aquaria (80 X 60 X 30 cm) filled with 90 L., de-chlorinated fresh water and aerator. The water temperature, dissolved oxygen, pH, ammonium (NH_4) and nitrite were measured and were 27 ± 2°C, 5.4 mg/l, 7.2, 0.20 mg/l and 0.02mg/l, respectively.

Fish diets and feeding:

A basic diet containing crude protein, crude lipid, vitamins and minerals met the basic dietary requirements of *O. niloticus*. We obtained the pure dried S. platensis (*Arthrospira platensis*) tablets from Lake Heath Products Co., Ltd. Liyang City, Jiangsu Province, China. Each tablet was grounded into powder before applying .

Experimental design:

Fish were separated into 4 equal triplicate groups. Each group included 20 fish. All fish were fed their respective diets at a point of 5% of body weight four times daily for 2 months.

Fish diet and feeding:

Diet number one is considered as control diet (no spirulina addition), while diet 2, 3, 4 supplemented diets contained Spirulina at 0.5, 1 and 1.5 %, respectively. Feedstuffs used in diets formulation were analyzed for dry matter, crude protein, ether extract and crude fiber according to the standard procedures of the A.O.A.C (1990) and the data were shown in Table 1.

Isocaloric and isonitrogenous diets were prepared at Fish Research Center, Faculty of Veterinary Medicine, Zagazig University, Egypt. The calories level is 2940 kcal/kg ME and crude protein is 30.80% in the form of dry pellets .the diet was formulated to meet the nutrient requirements of *Oreochromis niloticus* as set by NRC (1993) and shown in Table 2.

Growth performance parameters:

Fish were weighted at the start, every 14 days and the end of the experiment. The growth performance, Mean body weight, Body gain and feed conversion ratio were determined (Siddiqui *et al.,* 1988). Body gain percent (Jauncay and Ross, 1982) and specific growth rate % (Pouomonge and Mbonglang, 1993) were determined.

Health status:

The condition factor was determined according to Gjederm and Gunnes (1978). For evaluation of health condition of the fish during the period of the experiment, different reflexes was detected (escape, defensive, tail and ocular) were regularly observed according to Lucky (1977).

Blood sample collection:

Caudal blood vessels were the source of collection of blood. The collected blood was put in plastic Eppendorf tubes for serum samples preparation without anti-coagulant in syringe then centrifuged (3,000 r.p.m. for 15 min). The serum samples were collected and stored immediately in deep freezer (-20ºC) until use (Aly *et al*., 2008).

Immunological parameters evaluation:

Assay procedure for of IgM:

Immunoglobulin M (IgM) was determined using ELISA Kit. Catalog No. CSB-E12045Fh (96 test). (CUSABIO BIOTECH CO., Ltd).

Lysozyme determination:

The lysozyme activity was assessed according to method described by Parry *et al.,* 1965.

Challenge test:

After 2 months, twenty fish which fed on 1% Spirulina enriched diets and twenty fish from control group were collected then challenged with pathogenic

strain of *Areomonas hydrophila* (10^8 cfu mL^{-1}) that had previously isolated from moribund fish and confirmed its pathogencity (Talpur and Ikhwanuddin, 2012). Fish were inoculated by intraperitoneal injection with 0.1 ml of pathogenic strain of *Areomonas hydrophila* Collins *et al.* (1991). Infected fish were observed for any changes for a period of 15 days and mortality was verified by re-isolating the microorganism from internal organs of dead fish.

Statistical analysis:

Data in this study were statistically analyzed for variance ANOVA, LSD (Least significant difference) according to (Snedecor and Cochran, 1982). Comparing differences among treatment means were detected using Duncan's multiple range tests (Duncan, 1995). Data were presented as mean ± SE and significance was declared at ($P < 0.05$).

RESULTS AND DISCUSSION

Growth performance:

Our study revealed that the growth performance expressed as total final BW, body gain, body gain % and specific growth rate % were significant ($P < 0.05$) increase in groups fed Spirulina enriched diets versus the control group, the higher mean value level was in group 3 and 4 fed Spirulina enriched diets as shown in Table 3. While a significant ($P < 0.05$) decrease in the total feed conversion ratio in all supplemented groups than those fed diet 1, with lower mean value level in groups 3 and 4 fed Spirulina enriched diets. There was no significantly a ($P > 0.05$) difference in daily feed intake in groups 2 and 3, while group 4 showed a significant ($P < 0.05$) decrease than those fed the control diet. These results are also in accordance with Takeuchi *et al.* (2002) who reported that supplement S. platensis powder in diet of striped jack, the feed conversion ratio and growth rates were improved. Also, larval tilapia body weight was improved by feeding on raw S. platensis at rate of 30% (on a dry basis) of uni-feed (Lu *et al*., 2002). *O. niloticus* were showed maximum growth performance and better feed utilization at 5.0 g fresh culture of S. platensis /kg diet (Abdel-Tawwab and Ahmad, 2009) or at 10 g/kg diet of dry culture of S. platensis (Ibrahim *et al.*, 2013). The positive growth promoting effects of Spirulina may be due to good source of protein for animal feed, being

containing high amounts of vitamins and minerals (Duncan and Klesius, 1996); their positive immunostimulating effect or lack of cellulose from the cellular structure of Spirulina make it digestible easily, improving both of appetite, feed intake; nutrient digestibility; the health and immunity of fish against infections (Nakono *et al.*, 2003). On the contrary, Ungsethaphand *et al.* (2010) recorded that the growth performance of tilapia weren't affected by S. *platensis* supplementation.

Health status:

Condition factor and survival rate were shown in Table 4. Both of condition factors and survival rate of the fish were ideal in all groups. These results were matched with Jun *et al.* (2002) who confirmed the high beneficial effect of using Spirulina enriched diets on *O. niloticus* .

Immunological response:

Dietary supplements had significantly ($P < 0.05$) increased immunity of all groups compared to the control group as shown in Table 5. Lysozyme is one of the defensive factors against invasion by microorganisms. The level of lysozyme was significant in all fish groups fed on basal diet supplemented with S. platensis. Results were in accordance with Ibrahim *et al.* (2013) who recorded that 10% spirulina in fish fed with showed improving in haematocrit values. White shrimp L. *vannamei* that fed on hot-water extract of S. platensis had improved innate immunity as lysozyme and reduce the frequency of infection by *V. alginolyticus* infection (Tayag *et al.*, 2010). The presence of C-phycocyanin which was found in the Spirulina alga may be the reason of immunity capacity (Vonshak, 1997).

Infection with *Aeromonas hydrophila*:

Table 6 showed the survival and mortality rate of *O. niloticus* infected with pathogenic strain of *A.hydrophila*. The results indicated that the highest survival rate was in fish fed 1% Spirulina enriched diets comparing with control group. Control group showed signs of hemorrhages and loss of scales; while fish fed 1% Spiulina enriched diet wasn't shown any gross lesion. This may be due to the highly beneficial effect of Spiulina in improving the immune

response of fish against the infection with A. *hydrophila.* These results are completely agreed with Ibrahim *et al.* (2013) who observed that following the challenge infection using *Pseudomonas fluorescens* the mortality rate was significantly lower in all S. platensis supplemented groups in comparing with the control throughout the experimental period. Better survival rate could be mentioned that Spirulina algae has carotenoids at which fish health and ability to fight off infections through the reduction of stress levels specifically was improved (Duncan and Klesius, 1996) or improved immune status, such as phagocytosis, superoxide and cytokines production in common carp (Watanuki *et al.*, 2006).

CONCLUSIONS

It could be concluded that application concentration of dried S. *platensis* at 10 g/kg of diet for 2 months in feeding of *O. niloticus* is highly beneficial in improving growth performance, health conditions and the non-specific immunity of *Oreochromis niloticus*, as well as its protection effect to challenge by *Aeromonas hydrophila*. It is recommended to supplement Spirulina in the diet of *O. niloticus* due to its highly excellent effect in both growth and protection against infection with *A. hydrophila*.

Table 1. Showed proximate chemical composition of feedstuffs used in formulation of the experimental diets (analyzed).

Ingredient	Nutrient (% as fed basis)					
	DM	CP	EE	CF	Ash	NFE (Calculated)
Yellow corn	89.00	8.75	3.70	2.20	1.20	73.15
Spirulina platensis	94.80	62.10	3.20	3.00	10.00	21.70
Wheat flour	88.90	12.80	2.50	1.50	1.60	70.50
Soybean meal	90.00	43.70	1.80	6.10	6.50	31.90
Fish meal	94.80	63.40	8.70	0.7	20.70	1.30
Poultry by-product meal	92.60	60.30	12.70	2.10	14.70	2.80

(DM= Dry matter, CP= Crude protein, EE= Ether extract, CF= Crude fiber and NFE= Nitrogen free extract).

Table 2. Showed chemical composition of the experimental diets.

Ingredients (%)	Experimental diet			
	Control	Spirulina enriched diets		
		% ٠.٥	% ١	% ١.٥
Yellow corn	35	30	30	25
Spirulina	-	0.5	1	1.5
Wheat flour	10	10	10	10
Soybean meal, %٤٤	18	18	18	18
Fish meal, %٦٠	16	15.5	15	14.5
Poultry by-product meal	14	14	14	14
Vegetable oil	5.5	5.5	5.5	5.5
Vitamin and mineral mixture*	1.5	1.5	1.5	1.5
Calculated composition				
DM, %	٨٤.٢٨	٨٣.٨١	٨٣.٣٣	٨٢.٨٦
CP, %	٣٠.٧٩	٣٠.٧٩	٣٠.٧٨	٣٠.٧٨
EE, %	٩.٩٢	٩.٩٠	٩.٨٧	٩.٨٤
CF, %	٢.٤٠	٢.٤١	٢.٤٢	٢.٤٣
Ash, %	٧.٠٩	٧.٠٤	٦.٩٨	٦.٩٣
NFE, %	٣٨.٩٩	٣٩.١٠	٣٩.٢٠	٣٩.٣٠
DE, Kcal/ kg diet**	٢٩٤٤.٤١	٢٩٤٦.٤٨	٢٩٤٩.٠١	٢٩٥١.٦٢

* Vitamin and Mineral mixture (alfakema):- Each 1 kg contains:-Vit. A 580000 I.U, vit.D3 8600 I.U, vit.E. 720 mg, vit. K3 142 mg, vit C 0.1 mg, vit B1 58 mg, vit B2 34 mg, vit. B6 34 mg , vit.B12 58 mg , Folic acid 86 mg , Pantothenic acid 8 mg , Manganese sulfate 65 mg , Zinc methionine 3000 mg , Iron sulfate 2000 mg , Copper sulfate 3400 mg , Cobalt sulfate 572 mg , Sodium selenite 25 mg, Calcium iodide 25 mg, Calcium carbonate (Carrier substance) till 1000 gm.

** digestible energy calculation based on values of protein 3.5 kcal/gm, fat 8.1 kcal/gm, NFE 2.5 kcal/gm **(Santiago *et al.*, 1982).**

(DM= Dry matter, CP= Crude protein, EE= Ether extract, CF= Crude fiber and NFE= Nitrogen free extract**).**

Table 3. Showed effect of dietary supplementation with different levels of Spirulina on growth performance of *O. niloticus***.**

Ingredients (%)	Experimental diet			
	Control	Spirulina enriched diets		
		0.5 %	1 %	1.5 %
Initial body weight, g	33.71 ±0.77	34.15 ±0.69	33.98 ±0.88	33.99 ±0.70
Final body weight, g	42.71 ±0.39^{c}	44.85 ±0.29^{b}	47.21 ±0.22^{a}	47.58 ±0.54^{a}
Body weight gain, g	9.00 ±0.68^{b}	10.70 ±0.85^{ab}	13.23 ±107^{a}	13.58 ±1.14^{a}
Body weight gain, %	26.82 ±2.61^{b}	31.45 ±3.11^{ab}	39.16 ±4.15^{a}	40.12 ±4.17^{a}
Specific growth rate, %	0. 40 ±0.03^{b}	0.46 ±0.04^{b}	0.56 ±0.05^{a}	0.57 ±0.05^{a}
Feed consumption, g	33.28 ±0.26^{b}	33.70 ±0.28^{b}	34.08 ±0.23^{b}	35.85 ±0.30^{a}
Feed conversion ratio	3.74 ±0.29^{a}	3.19 ±0.29^{ab}	2.61 ±0.22^{b}	2.67 ±0.26^{b}

^{abc} Mean in the same row with different superscripts are significantly different at ($P < 0.05$).

Table 4. Showed effect of dietary supplementation with different levels of Spirulina on condition factor and survival rate of *O. nilioticus*.

Ingredients (%)	Experimental diet			
	Control	Spirulina enriched diets		
		0.5 %	1 %	1.5 %
Initial body length, cm	11.22 ±0.66	11.35 ±0.69	11.44 ±0.88	11.35 ±0.70
Final body length, cm	13.21 ±0.39	13.55 ±0.29	13.95 ±0.22	13.75 ±0.54
Condition factor (K)	1.85	1.8	1.73	1.83
Survival rate, %	95.20 ± 0.0	96.30 ± 0.14	97.30 ± 0.12	97.20 ± 0.14

Table 5. Showed effect of dietary supplementation with different levels of Spirulina on immune status of *O. nilioticus*.

Ingredients (%)	Experimental diet			
	Control	Spirulina enriched diets		
		0.5 %	1 %	1.5 %
Initial IgM value (µg /ml)	21.53 ±0.28	21.16 ±0.33	21.32 ±0.44	21.00 ±0.76
Final IgM value (µg /ml)	27.50 ±0.57^{d}	30.20 ±0.15^{c}	41.40 ±0.66^{a}	38.41 ±0.01^{b}
Initial lysozyme value (µg /ml)	14.00 ±0.25^{a}	13.50 ±0.57^{ab}	12.88 ±0.38^{ab}	12.26 ±0.69^{b}
Final lysozyme value(µg /ml)	17.83 ±0.33^{d}	20.00 ±0.26^{c}	30.33 ±0.44^{a}	27.50 ±0.22^{b}

^{abcd} Mean in the same row with different superscripts are significantly different at ($P < 0.05$).

Table 6. Showed results of experimental infection of *O. niloticus* with *Aeromonas hydrophila*.

Group N=20	Survival percentage (%)	Mortality rate percentage (%)
Control	30	70
Spirulina enriched diets % ١	85	15

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1. A

1. B

Fig. ١. A. *O. niloticus* inoculated with *A.hydrophila* ،showing no signs.

1. B. *O. niloticus* infected with *A.hydrophila*, showing signs of hemorrhages at dorsal and caudal fins with loss of scales.

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تاثير إضافة فطر السبيرولينا في عليقة أسماك البلطي النيلي علي النمو والصحة والتأثير الوقائي في حالة الأصابة بالإيروموناس هيدروفيلا

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الملخص العربى

تم عمل دراسة علي تاثير إضافة فطر السبيرولينا علي نمو ومناعة ومقاومة أسماك البلطي النيلي لميكروب الإيروموناس هيدروفيلا. أستخدم عدد ٢٤٠ من أسماك البلطي النيلي بمتوسط وسط ٣٣±١ جم حيث تم تقسيم الأسماك الي أربع محموعات بثلاث تكرارات. تم تغذية الأسماك علي أربع علائق :١- العليقة الضابطة ٢- عليقة ضابطة بإضافة ٠.٥ % من فطر السبيرولينا ٣- عليقة ضابطة بإضافة ١% من فطر السبيرولينا ٤- عليقة ضابطة بإضافة ١.٥% من فطر السبيرولينا لمده شهرين. وبعد ذلك تم عمل عدوي بميكروب الإيروموناس هيدروفيلا للأسماك المغذاه علي العليقة الضابطة والأسماك المغذاه علي ١% من فطر السبيرولينا.

وأظهرت النتائج بأن الأسماك المغذاه علي العليقة الضابطة وفطر السبيرولينا خاصة ١% من فطر السبيرولينا *أدت الى* زياده في معدلات النمو وتحسن واضح في الحاله المناعية مقارنة بالأسماك المغذاه علي العليقة الضابطة فقط . كما أوضحت النتائج أن الأسماك المغذاه ١% من فطر السبيرولينا *أدت الى* مقاومة واضحة لميكروب الإيروموناس هيدروفيلا.

من النتائج السابقة يتضح بأنة من المفضل إضافة ١% من فطر السبيرولينا علي العليقة الضابطة لأسماك البلطي النيلي لتحسين النمو والحالة المناعية ومقاومة الأسماك لميكروب الإيروموناس هيدروفيلا.