Slov Vet Res 2018; 55 (Suppl 20): 157–63
DOI 10.26873/SVR-642-2018

Original Research Article

EVALUATION OF *SPIRULINA PLATENSIS* ENRICHED DIET ON GROWTH PERFORMANCE, BIOCHEMICAL PARAMETERS AND IMMUNE RESPONSE OF *OREOCHROMIS NILOTICUS*

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Abstract: Two hundred and twenty five *Oreochromis niloticus* with average body weight 36±1 g, were distributed randomly into five groups in triplicates. The control group was fed on basal diet, while the other groups were supplemented with various levels of Spirulina platensis (0.5, 1, 1.5 and 2%) for 90 days. The growth performance, biochemical parameters (Alanine aminotransferase activity, Aspartate aminotransferase, Creatinine and cortisol), antioxidant effect (Reduced Glutathion enzyme), IgM and lysozyme were determined. Also, investigating the differences between fish fed the control diet and diet supplemented with Spirulina (1%) against the infection with pathogenic strain of *Aeromonas hydrophila*. The final body weight, gain percent and specific growth rate was significantly (P<0.05) increased in fish fed on supplemented diet with Spirulina comparable with that fed on the control diet. The highest final body weight (49.60±0.52g), body gain (11.70±1.04g) and body gain (30.87±4.10%) were recorded in fish fed on diet supplemented with 2% Spirulina. Biochemical, immunological parameters and antioxidant enzyme (GSH) were improved in the group supplemented with Spirulina in comparing with fish fed on basal diet. The best result was recorded in-group fed on Spirulina supplemented diet by level of 1% at which the levels of IgM was 42.40 ±0.66 µg /ml, lysozyme level was 31.33 ±0.44 µg /ml and the level of GSH was 11.7 ±0.39 mg/g tissues. Moreover, Spirulina supplemented with (1%) in diet enhanced fish protection against *A. hydrophila* infection. The survival rate was (80%) in fish fed on diet supplemented with 1% Spirulina.

Key words: Spirulina; *Oreochromis niloticus*; growth; immunity

Introduction

Recently, aquaculture is one of the fastest growing in our world as it has a high level of protein, which is considered the lowest price in comparing with other sources of protein (1). For human, fish is one of the important animal proteins. Nowadays, we all encourage the culturing of fish through many facilities to provide protein all over the world. fish protein is a very excellent source of protein in replacing of red meat .fish flesh contains all of minerals , iodine ,potassium ,iron copper , and vitamin D, A with favorable levels (2).

Vaccines, antibiotics and chemical therapy are used to protect fishes against the infection.

Received: August 2018
Accepted for publication: September 2018

The excretive use of antibiotics will cause severe damage to fish, disease resistant, severe environmental problems and hazards in food safety (3). also , presence of different causes of infection to fish will interfering with vaccine manufacture (4) so, it is important to use natural plants to improve immunity of fish and to enhance the production to the highest level. The response of fish to infection is occur through two mechanisms specific and nonspecific also, it determined through immune response of fish to infection (5) .To protect fish against the infection , the best way was done by improving of its immunity . Many of natural plants and algae including spirulina used to increase the immunity of fish (6).

Spirulina (*Spirulina platensis*) is marine algae, which its color is green and blue. It grows in lakes, which is rich with carbon. Cyanobacterium is traditionally produced for human, as it is rich with high quality protein and a lot of nutrients product as vitamins, minerals and essential fatty acids (7). Nowadays *S. platensis* are widely used for improving nutrient composition and physiological response to stress in many fish species (8). Recently, *S. platensis* has a powerful effect in improvement the immunity of Carp fish (9). Therefore, the aim of the current study was to investigate the effects of dietary supplementation with dried *S. platensis* at different doses on the immune parameters, growth performances and the resistance to the pathogenic *Aeromonas hydrophila* of *O. niloticus*.

Material and methods

Fish and the experimental design

Two hundred and twenty five apparently healthy live *Oreochromis niloticus* with an average body weight 36±1.0 g acquired from Abassa Fish Farm at Sharkia Governorate. They were kept in glass aquaria (80X60X30 cm) provided with 90 L de-chlorinated fresh water and aerator with 27 ± 2°C water temperature, 5.4 mg/l dissolved oxygen, 7.2 pH, 0.20 mg/l ammonium (NH_4) and 0.02mg/l nitrite. Fish were separated into five equal triplicate groups, each included 15 fish. All fish were fed their respective diets at a point of 3.5% of body weight four times daily for 3 months. The study was approved by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University.

Fish of the control group were fed on basal diet (the basic dietary requirements of *O. niloticus* (10) (Table 1). Other groups were fed on diet supplemented with different level of spirulina (0.5, 1, 1.5 and 2%) (Pure dried *S. platensis* (*Arthrospira platensis*) tablets, Lake Heath Products Co., Ltd. Liyang, Jiangsu, China; each tablet was grounded into powder before used). The diet was analyzed for dry matter, crude protein, ether extract and crude fiber (11). 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.

Table 1: The chemical composition of the experimental diets used in feeding of *O. niloticus*

Ingredients (%)	Experimental diet				
	Control	Spirulina enriched diets			
		0.5%	1%	1.5%	2%
Yellow corn	35	35	35	35	35
Spirulina[1]	-	0.5	1	1.5	2
Wheat flour	10	10	10	10	10
Soybean meal, %44	18	18	18	18	18
Fish meal, %60	16	15.5	15	14.5	14
Poultry by-product meal	14	14	14	14	14
Vegetable oil	5.5	5.5	5.5	5.5	5.5
Vitamin and mineral mixture*	1.5	1.5	1.5	1.5	1.5
Calculated composition					
DM, %	84.28	83.81	83.33	82.86	82.39
CP, %	30.79	30.79	30.78	30.78	30.77
EE, %	9.92	9.90	9.87	9.84	9.81
CF, %	2.40	2.41	2.42	2.43	2.45
Ash, %	7.09	7.04	6.98	6.93	6.88
NFE, %	38.99	39.10	39.20	39.30	39.40
DE, Kcal/ kg diet**	2944.41	2946.48	2949.01	2951.62	2954.23

* 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 **(12).**
(DM= Dry matter, CP= Crude protein, EE= Ether extract, CF= Crude fiber and NFE= Nitrogen free extract).
[1] Analyzed composition for *Spirulina platensis* includes (94.80% DM, 62.10% CP, 3.20% EE, 3% CF, 10% Ash and 21.70% NFE).

Growth performance and health status

Fish were weighted at the start, every 14 days and the end of the experiment. The final body weight, body gain (g), body gain percent, specific growth rate %, food consumption (g), feed conversion ratio and condition factor were determined (13-16). Regarding the assessment of fish health status during the experimental period, different reflexes was detected (escape, defensive, tail and ocular) were regularly observed (17).

Blood and liver samples 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 collected serum were stored immediately in deep freezer (-20ºC) until use (18). At the start and end of the study one and half gram of livers were taken and homogenized in 5ml cold 20 mM HEPES buffer, pH 7.2, containing 1mM EGTA, 210 mM mannitol and 70mM sucrose per gram tissue. Homogenates were centrifuged at 10.000 x g for15 minutes at 4 °C and the supernatant was collected and stored at-80°C until further determination of reduced Glutathione enzyme (GSH).

Immunological and biochemical parameters

Immunoglobulin M (IgM) was determined using ELISA Kit (Catalog No. CSB-E12045Fh (96k test). CUSABIO BIOTECH CO., Ltd). The lysozyme activity was measured using the turbidity assay (19). Alanine Aminotransferase Activity (ALT) and Aspartate Aminotransferase (AST) were determined (Spectrum Kits, Egyptian Company for Biotechnology, Cairo, Egypt (REF: 265 002 and 261 002, respectively) (20). Creatinine was carried out according to method mentioned by Husdan and Rapoport (21). The **s**erum cortisol was determined by ELISA using Microtiterstrip DIAMED CORTISOL ELISA kit. Reduced glutathione (GSH) was evaluated in the liver homogenate (kit, Cayman, Cat. No. 703002, Cayman, USA) (22).

Challenge test

After 3 months, fifteen fish, which fed on 1% Spirulina enriched diets, and other fifteen fish from control group were collected then challenged with pathogenic strain of *Areomonas hydrophila* (10^8 cfu mL^{-1}) obtained from animal health institute (23). Fish were inoculated by intraperitoneal injection with 0.1 ml of pathogenic strain of *Areomonas hydrophila* according to Collins et al. (24). Infected fish were observed for any changes and the mortalities of all replicates were calculated for a period of 15 days. The mortality was verified by re-isolating the microorganism from internal organs of dead fish. The fish were counted at the end of experiments to determine the survival percentage according to the following formula:

Survival % = (Final number of tested fish/Initial number of tested fish) x 100.

Statistical analysis

Data were statistically analyzed using one way ANOVA, LSD (Least significant difference) based on Snedecor and Cochran (25). Comparing differences among different means were detected with Duncan's multiple range tests (26). Data were showed as mean ± SE and the significance was considered at ($P < 0.05$).

Results

Growth performance and health status

Regarding the growth performance**,** both of total final body weight**,** body gain (g), body gain %, specific growth rate %, feed consumption and feed conversion ratio were determined. The results were demonstrated in Table (2) which revealed that fish fed on supplemented diet with Spirulina showed significant ($P<0.05$) improve in growth performance in comparing with fish which fed on basal diet. There were no significant ($P<0.05$) changes in both of condition factor and survival rate among the fish during the experimental period (Table, 2).

Table 2: The effect of dietary supplementation with different levels of Spirulina on growth performance, condition factor and survival rate of *O. niloticus*

Ingredients (%)	Experimental diet					
	Control	Spirulina enriched diets				P value
		0.5%	1%	1.5%	2%	
Initial body weight, g	36.71±0.77	36.15±0.69	37.98±0.88	37.99±0.70	37.90±0.71	0.545
Final body weight, g	44.71±0.39^{c}	45.85±0.29^{b}	49.21±0.22^{a}	49.58±0.54^{a}	49.60±0.52^{a}	0.011
Body weight gain, g	8.00±0.66^{b}	9.70±0.85^{ab}	11.23±107^{a}	11.59±1.14^{a}	11.70±1.04^{a}	0.022
Body weight gain, %	21.79±2.60^{b}	26.83±3.11^{ab}	29.57±4.15^{a}	30.51±4.17^{a}	30.87±4.10^{a}	0.020
Specific growth rate, %	0. 33±0.03^{b}	0.40±0.04^{b}	0.43±0.05^{a}	0.44±0.05^{a}	0.45±0.04^{a}	0.002
Feed consumption, g	30.25±0.26^{b}	31.70±0.28^{b}	32.08±0.23^{b}	33.85±0.30^{a}	34.00±0.30^{a}	0.002
Feed conversion ratio	3.78±0.29^{a}	3.26±0.29^{ab}	2.85±0.22^{b}	2.92±0.26^{b}	2.90±0.25^{b}	0.002
Initial body length, cm	11.22±0.66	11.35±0.69	11.44±0.88	11.35±0.70	11.44±0.65	0.935
Final body length, cm	13.21±0.39	13.55±0.29	13.95±0.22	13.75±0.54	13.94±0.33	0.432
Condition factor (K)	1.85	1.8	1.73	1.83	1.72	--
Survival rate, %	95.20± 0.0	96.30± 0.14	97.30± 0.12	97.20± 0.14	96.21±0.22	1

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

Immunological and biochemical parameters

Table (3) showed the different level of IgM and Lysozyme to fish fed on dietary supplements with Spirulina and basal diet. Both of IgM and Lysozyme were significantly ($P < 0.05$) improved in all fish fed on dietary supplements with Spirulina compared to the control group. Dietary supplementation with 1% Spirulina showed significantly increase in both of IgM and Lysozyme in comparing with other groups.

Regarding to the impact of dietary supplementation with different level of Spirulina on different biochemical blood parameters of *O. niloticus*, there were no significant variation (P<0.05) between the control group and other groups.

From Table 3, it was clear that significant (P<0.05) increasing level of GSH enzyme in all group fed with supplemented diet with Spirulina in comparing with fish fed on basal diet.

Challenge experiment

The highest survival (80%) rate was recorded in fish fed on 1% Spirulina enriched diets comparing with fish fed on basal diet group (33%).

Table 3: The effect of dietary supplementation with different levels of Spirulina on immune status, blood parameters and liver GSH of *O. nilioticus*

Ingredients (%)	Experimental diet					P value
	Control	Spirulina enriched diets				
		0.5%	1%	1.5%	2%	
Initial IgM value (µg /ml)	22.53±0.28	22.16±0.33	22.32±0.44	22.00±0.76	22.16±0.33	0.692
Final IgM value (µg /ml)	28.50±0.57^{d}	31.20±0.15^{c}	42.40±0.66^{a}	39.41±0.01^{b}	39.23±0.02^{b}	0.011
Initial lysozyme value (µg /ml)	15.00±0.25^{a}	14.50±0.57^{ab}	13.88±0.38^{ab}	13.26±0.69^{b}	13.85±0.52^{ab}	0.026
Final lysozyme value(µg /ml)	18.83±0.33^{d}	21.00±0.26^{c}	31.33±0.44^{a}	28.50±0.22^{b}	27.95±0.24^{b}	0.002
Initial ALT value (µg /ml)	17.21±0.36	16.96±0.26	16.8±0.35	17.23±0.30	16.95±0.16	0.945
Final ALT value (µg /ml)	16.53±0.06^{a}	15.80±0.65^{a}	15.74±0.24^{a}	16.71±0.14^{a}	16.46±0.54^{ab}	0.011
Initial AST value (µg/ml)	17.65±0.42	17.22±0.55	17.33±0.32	17.14±0.46	17.02±0.26	1
Final AST value (µg/ml)	16.95±0.22^{a}	17.01±0.35^{a}	17.12±0.61^{a}	16.96±0.45^{a}	17.01±0.35^{a}	0.017
Initial liver GSH (mg/g tissue)	9.27±0.35	8.97±0.67	9.01±0.48	9.17±0.53	8.84±0.73	0.547
final liver GSH (mg/g tissue)	9.05±0.55^{c}	8.63±0.52^{c}	11.7±0.39^{a}	10.52±0.55^{b}	10.94±0.64^{b}	0.002
Initial Creatinine (mg/dl)	0.22±0.32	0.21±0.41	0.19±0.52	0.22±0.12	0.21±0.22	0.390
Final Creatinine (mg/dl)	0.18±0.12^{a}	0.18±0.32^{a}	0.17±0.42^{a}	0.19±0.27^{a}	0.17±0.35^{a}	0.303
Initial cortisol value (µg/ml)	6.22±0.25	6.34±0.35	6.55±0.51	6.42±0.43	6.21±0.22	.501
Final cortisol value (µg/ml)	5.21±0.27^{a}	5.33±0.21^{a}	5.21±0.31^{a}	5.12±0.52^{a}	5.22±0.15^{a}	0.112

^{abcd} Mean in the same row with different superscripts are significantly different at (P < 0.05).
IgM= Immunoglobulin M, ALT= Alanine Aminotransferase Activity, AST= Aspartate Aminotransferase and GSH= Reduced Glutathione enzyme

Discussion

Growth performance and health status

The current study revealed an improvement of the growth performance of fish supplemented with *Spirulina platensis*. These results are in accordance with Takeuchi et al. (8) who stated that the feed conversion ratio and growth rates were improved with *S. platensis* powder supplementation in diet of striped jack. Also, larval tilapia body weight was improved by feeding on raw *S. platensis* with 30% (dry basis) of uni-feed (27). *O. niloticus* were showed maximum growth performance and better feed utilization with 5g fresh culture of *S. platensis* /kg feed (28) or at 10 g/kg diet of dry culture of *S. platensis* (29). The growth promoting effects of Spirulina may be due to its content of high levels of vitamins and minerals that provide a superior protein used for animal feeding (30). The positive immunostimulating effect and the low amount of cellulose in spirulina cellular structure make its digestible simply, improving both of appetite, feed intake; nutrient digestibility; the health and immunity of fish against infections (31). In contrast, the growth performance of tilapia weren't affected by S. *platensis* supplementation (32). The current results of the health status were matched with Jun et al. (33), who confirmed the high beneficial effect of using Spirulina enriched diets for feeding of *O. niloticus*.

Immunological and biochemical parameters

The findings of this study were similar to Ibrahim et al. (29) who recorded 10% spirulina in fish fed showed improving in its hematocrit

values. White shrimp *Litopenaeus vannamei* (*L. vannamei*) fed on hot-water extract of *S. platensis* improved innate immunity (lysozyme) and reduce the frequency of infection by *Vibrio alginolyticus* (34). The existence of *C-phycocyanin* which was found in Spirulina alga may be the reason of immunity capacity (35). The detected levels of plasma AST and ALT is of significant diagnostic importance in fish. They reflect the general nutritional condition and the integrity of vascular system and liver function. The current findings were matched with Upasani and Balaraman (36) who stated that using of Spirulina in feeding of fish have a powerful effect in decreasing liver and kidney lipid peroxidation. In addition, it makes increasing of antioxidant enzymes in fish as it contains antioxidants substance as carotene, minerals, vitamins, protein, carbohydrates and lipids.

Challenge experiment

This indicted the high beneficial effect of fed fish with supplemented diet with1% Spirulina in raising the immune of the fish to a great extent that make fish can withstand the infection. The results are completely agreed with Ibrahim et al. (29) who observed that *S. platensis* supplementation significantly reduced the mortality rate post challenge infection with *Pseudomonas fluorescens.* Better survival rate could be mentioned that Spirulina algae has carotenoids that improved fish health and capability to resist infections through lessening the stress (30) or improved immune status, such as phagocytosis, producing superoxide and cytokines in common carp (37).

Conclusion

From our study, it could be concluded that the using of 10g/kg dried *S. platensis* in diet for 3 months as it have an excellent effect in improving of growth rate and immunity of *O. niloticus* . Also, feeding *O. niloticus* with diet supplemented with S. *platensis* (1%) will strength it against the infection with pathogenic strain of *A.hydrophila* through improving immunity.

Conflict of interest

The authors declare no conflict of interest.

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