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EFFECT OF SPRULINA PLATENSIS ON THE IMMUNE RESPONSE OF CULTURED OREOCHROMIS NILOTICUS

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ABSTRACT

The use of microalgae in the field of fish feeding is still operated in a small scale. This study was conducted to evaluate the effects of using the microalga *Spirulina platensis* powder as a feed additive in fish diets. So, we use a total number of 120 apparently healthy *Oreochromis niloticus*, with average body weight of fish (50±5g) were obtained from Barseek fish farm at Behera Governorate.

The present work was designed to investigate the different immunostimulant effects of Spirulina on some cultured freshwater fish including serum proteins, (Lymphocytes & monocytes) as well as phagocytic activity, index and the level of antibody titer, relative level of protection .

For these investigations four experimental diets were carried out by addition of *Spirulina platensis* powder to balanced fish diet .

- **1st group : fed on 1st diet which is formed of fish diet with addition of 2.5% *Spirulina platensis* powder to it.**
- **2nd group : fed on 2nd diet which is formed of fish diet with addition of 5% *Spirulina platensis* powder to it.**
- **3rd group : fed on 3rd diet which is formed of fish diet with addition of 10 % *Spirulina platensis* powder to it.**
- **4th group : fed on 4th diet which is formed of fish diet without addition of *Spirulina platensis* powder to it (Control group).**
- **The most important results of this study include :**

1. **There was a tendency for the WBCs values had the highest values during addition of Spirulina also there was increasing in lymphocyte and monocytes in groups fed on Spirulina than the control groups.**
2. **Fish groups supplemented with Spirulina showed increased of phagocytic activity and phagocytic index until the 4th weeks from feeding Spirulina.**
3. **In the present work, the significant increase in albumin, globulin and total protein and increase of albumin/globulin (A/G) ratio in the groups fed on Spirulina than control group**
4. **The antibody titers in all Spirulina supplemented groups were higher than the control feed on basal diet where ranged between 2±0.1, 3±0.1, 4±0.3 and 4±0.3 during 1st, 2nd, 3rd and 4th weeks respectively and at the end of the 4th week, the antibody titers ranked in groups**

where the 10% Spirulina supplemented group come in the first rank, followed by 5% Spirulina supplemented group, then 2.5% Spirulina supplemented group while the control group came in the last rank in values.

5. **The potency of bacterin was examined by calculating the relative level of protection (RLP). In this study, we examined the disease resistance in Spirulina - treated tilapia using the tilapia pathogen *Aeromonas hydrophila*.**

INTRODUCTION

Fish is considered the cheapest food article of high nutritive value. The commercial production of fish is a rapidly growing industry and concurrent with the growth, fish cultures are rapidly expanding allover the world ***(Jehan, 2001).***

Diets in aquaculture are based on conventional feedstuffs such as fish oil and fish meal, but these are very expensive. The future development of aquaculture will be greatly constrained by the availability of an alternative source of feed ingredients. The proportion of global fish meal production used in fish feeds has increased from 10% to 35% over the last 15 years. Predictions of fish meal requirements for the future are approximately 44% of the 10 years average global fish meal production ***(Naylor et al., 2000).*** Studies made on Spirulina demonstrated the usefulness of Spirulina for partial or complete replacement of fish meal in the diets of two Indian major carps, Catla ***(Catla catla)*** and rohu, ***(Labeo rohita) (Nandeesha et al., 2001***). Also, Spirulina can replace up to 40% of the fish meal protein in tilapia (***Oreochromis niloticus***) diets ***(Olvera-Novoa et al., 1998).***

Therefore the present work was conducted to fulfill the following objectives:-

- To evaluate the use of the microalgae ***Spirulina platensis*** as a fish meal replacement in practical diets for tilapia ***(Oreochromis niloticus)*** with the objective of evaluating its nutritional quality.
- To establish the immune-potentiating function of ***Spirulina (S. plantensis)*** in tilapia (***Oreochromis niloticus).***

MATERIAL AND METHODS

1- Fish :

A total number of 120 apparently healthy ***Oreochromis niloticus***, with average body weight of (50± 5g / fish) were obtained from Barseek fish farm at Behera Governorate.

2- Aquaria:

Fish were kept in prepared glass aquaria (90 x 50 x 35 Cm). These aquaria were used for holding the experimental fish throughout the period of the present study, (triplicate each treatment), supplied with chlorine free tap water according to ***(Innes, 1966)***.

3- Fish diets:

Fish were fed on a commercial fish diet containing 25% crude protein. The diet was daily provided at a fixed feeding ratio of 3% of body weight of fish as described by ***Eurell et al. (1978).***

The quantity of feed related to fish weight was adjusted through weekly weighing at early mourning before feeding. The daily amount of food was offered as two equal meals / day on two occasions over the day (At 9 AM and 12 PM). Moreover, the fish mortality was recorded daily and so, the quantity of food was decided.

Table (1): The ingredient composition (%) of the basal diet (without supplementation of *Spirulina platensis* powder).

Ingredients	%
Fish meal (72%)	27
Soybean meal (44%)	23
Ground yellow corn	35
Wheat bran	10
Binders *	2
Mineral mixture	1.5
Vitamin mixture	1.5

* Binders: Sodium carboxy methyl cellulose (high viscosity) according to ***Murai et al. (1986).***

4- Spirulina platensis:

a- Commercially purchased Spirulina:

S. Platensis used in the present study was obtained from Agent Chemical Laboratories, Redmond, WA, USA.

Four experimental diets were used to feed tilapia (***O. niloticus)***, diet + 0% algae, diet + 2.5 % ***S. platensis***, diet + 5 % ***S. platensis*** and diet + 10% ***S. platensis*** respectively according to the method described by ***(Promya and Traichaiyaporn, 2004).***

5- Pathogens:

The ***Candida albicans*** and Bacterial strain ***Aeromonas hydrophila*** strain (N5) strain was kindly supplied by department of poultry and fish diseases Fac. Vet. Med., Alexandria University which is used for phagocytic assay study.

METHODS

The design of the experiment of fedding *S. platensis* to tilapia (*O. niloticus*) was presented in table (2) :

Treatments (Groups)	Number of replicates	Number of fish treated with different levels of commercially purchased *S. platensis*
Fish diets + 2.5% ***S. platensis***	III	30
Fish diets + 5% ***S. platensis***	III	30
Fish diets + 10% ***S. platensis***	III	30
Fish diets + zero% ***S. platensis*** (Basal diet)	III	30

1- Blood sampling:

Blood samples were collected weekly (for 4 weeks) from the caudal vessels using disposable tuberculin syringe which were used for determination of the following haematological investigations: -

White blood cell (WBCs) were counted by haemocytometer. Differential leucocytic count: Blood film was prepared according to the method described by ***(Lucky, 1977)***. Phagocytic assay was determined according to ***Kawahara et al. (1991).*** Results were expressed as means ± S.E. and differences were evaluated by Student's t–test. - Phagocytic activity (PA) =Percentage of phagocytic cells containing yeast cells.

$$\text{Phagocytic index (PI)} = \frac{\text{Number of yeast cells phagocytized .}}{\text{Number of phagocytic cells}}$$

2- Serum sampling: -

Serum separated and the serum preparation was done for clinico-biochemical determination according to the method described by ***(Lied et al., 1975)*** and used for measuring of the following serum biomarkers (total protein and albumin). Serum total protein was determined according to ***(Doumas et al., 1981)*** using commercial kits produced by ***Pasteur, Lab***. Serum albumin was determined according to ***Reinhold (1953)*** using commercially available kits of Chemroy. Serum globulin was determined by subtract the total serum albumin from total serum protein according to ***(Coles, 1974)*.** Determination of serum albumin / globulin ratio: Determined by division of serum albumin value on serum globulin value according to the method implied by ***(Coles, 1986).***

3- Evaluation of the immuno-potentiation effect of Spirulina platensis against A. hydrophila bacteria: -

Table (3): Design of the experiment of evaluation of the immunopotency effects of feeding *S. platensis* against *A. hydrophila*:-

Number of fish	Treatments
10	* Vaccinated treated with 2.5 % ***S. platensis.***
10	Non vaccinated treated with 2.5 % ***S. platensis.***
10	* Vaccinated treated with 5% ***S. platensis.***
10	Non vaccinated treated with 5% ***S. platensis.***
10	* Vaccinated treated with 10% ***S. platensis.***
10	Non vaccinated treated with 10% ***S. platensis.***
10	* Vaccinated non treated group.
10	Non vaccinated non treated group.

For ***O. niloticus***, groups: -

- *Vaccination by bacterine occurs at zero day and booster at 14 day.
- Scarification at 7, 14, 21, 28 days.
- Number of sacrificated fish for each time was 3 fish / group.
- Total number of sacrificated fish in each group = 6 X 3 = 18 fish.
- Challenge by live bacteria occurs after 28 days post vaccination.
- After challenge the mortality rate and the postmortem changes were recorded.
- Duration of experiment 4 weeks till challenge.

5. a. Bacterine preparation:

A. hydrophila isolate was used in the bacterine preparation according to the method described by ***Badran (1990)***. The organism was inoculated in Trypticase Soya Broth (TSB) containing 3.7 % NaCl and incubated at 28 C for 24 hrs. Safety and sterility tests of the prepared bacterine were carried out according to ***Anderson and Conroy (1970)*** by inoculation in TSB and incubated at 28 C for 24 hrs. The preparation of bacterine for injection was carried out according to the method of ***Badran (1990).*** The formalin inactivated bacterial cells were mixed with an equal volume of 0.85% sterile saline. Bacterial number was adjusted to fit MacFarland's No. 2.

Specificity of death was determined by re-isolation of injected bacteria from freshly dead fish during the period of observation (One week) according to ***Soliman (1988).*** The potency of bacterine was evaluated by calculating the relative level of protection (RLP) by the following formula:

$$RLP = 1 - \frac{\text{\% mortality of vaccinated fish}}{\text{\% mortality of control}}$$

According to ***Newman and Majnarich (1982).***

8- Statistical analysis:-

The data of haematological & biochemical analysis of the treated fish to different concentrations of Spirulina and also the control groups were statistically analyzed using t-test, Duncan-test after ANOVA and simple correlation according to ***(SAS, 1987)*** .

RESULTS

Effect of Spirulina on WBCs , lymphocyte, monocyte and phagocytic activity and index in the blood of O. niloticus: The data presented in table (4) outlined the mean values of total white blood cells (WBCs) % in ***O.niloticus*** supplemented by Spirulina in the feed by different percentages. It was observed that the WBCs values of 10% Spirulina supplemented group had the highest value (25 and 27.67 x 10^4 cells / $Cm^{3)}$ at 2nd and 3rd week respectively, while the lowest value (22.33 x 10^4 cells / cm^3) at the end of the experiment.

The differential leucocytic counts had the highest values during Spirulina supplemented by 5% from the basal diet. It was found that the lymphocytes values were 50.00 ± 0.58 and 50.33 ± 0.33 during added Spirulina by 5% and 10% and 45.00 ± 0.55 in control group at the same time in the first week. How ever, the lymphocytes values was 47.33 ± 0.33 and 49.67 ± 0.33 during added Spirulina by 5% and 10% and 46.67 ± 0.33 in control group at the same time in the 4th week.

Results of phagocytic activity (PA) and phagocytic index (PI) between different weeks among different treatments **are shown in table (4):** Data revealed that the both PA and PI values had the highest record in 10% Spirulina supplemented group. It was found that, the PA values were 23.00 ± 0.58, 23.33 ± 0.33, 24.33 ± 0.33 and 23.00 ± 0.58 during 1st, 2nd, 3rd and 4th week of 10% Spirulina supplemented group . On the other hand, The PI values were 2.23 ± 0.12, 2.30 ± 0.10, 2.47 ± 0.09 and 2.37 ± 0.03 at the same time of the previous mentioned treatment. It worthy noted that the both PA and PI values were had the lowest values in control group feed on basal diet all over the period of the experiment.

Effect of Spirulina on serum proteins (Total proteins, albumin, and globulin and albumin/globulin ratio):-The main values of Albumin, globulin, total protein and Albumin/globulin ratio **are shown in table (5)**. these treated groups were presented namely; 2.5%, 5% and 10% Spirulina supplemented groups. Results showed that the albumin was highest values in 5% Spirulina supplemented group, while the globulin values were highest in 2.5% Spirulina supplemented group compared with control feed on basal diet group. Meanwhile, total protein values were highest in 5% Spirulina supplemented group . It worthy to be noted that the Albumin/globulin ratio was highest values in both 5% and 10% Spirulina supplemented groups during 2nd and 3rd weeks of the experiment .

Effect of Spirulina on antibody titration : Table (6) : discussed the antibody titers in all groups supplemented with Spirulina in the feed and control group feed on basal diet. The table showed that the antibody titers was the highest in 10% Spirulina supplemented group during all periods of vaccination program (four weeks) (7 ± 0.4, 6 ± 0.3, 6 ± 0.3 and 7 ± 0.4) during 1st, 2nd, 3rd and 4th weeks respectively. The data obtained in the present table are in general cleared that the antibody titers in all Spirulina supplemented groups were higher than the control feed on basal diet where ranged between 2 ± 0.1, 3 ± 0.1, 4 ± 0.3 and 4 ± 0.3 during 1st, 2nd, 3rd and 4th weeks respectively. At the end of the 4th week, the antibody titers ranked in groups where the 10% Spirulina supplemented group come in the first rank, followed by 5% Spirulina supplemented group, then 2.5% Spirulina supplemented group while the control group came in the last rank in values.

Effect of Spirulina on Relative level of protection : The mortality number and relative level of protection of ***O.niloticus*** among different treatments **are presented in table (7):** Data showed that both mortality rate and relative level of protection were clearly improved in Spirulina supplemented group in comparison to the basal diet fed group. From such data, it was observed that the relative level of protection were 100%, 90%, 90% and 40% in vaccinated fish and Spirulina supplemented groups with 2.5%, 5%, 10% and basal diet feed group respectively. On other hand, these percentages reached to 60%, 40%, 50% and zero % in the previous mentioned treatments but non vaccinated.

LIST OF TABLES

Table (4): Means ± S. E of WBCs , Lymphocyte, Monocyte and Phagocytic activity and Indx among different treatments of fish at different weeks.

Week	Concentration	N	WBCs	Lymphocyte	Monocyte	PA	PI
			Mean±Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error
1st Week	2.5 %	3	A 25.00±0.58	B 46.67±0.33	C 1.33±0.33	B 22.33±0.33	A 2.50±0.23
	5 %	3	AB 24.33±0.33	A 50.00±0.58	B 1.67±0.33	B 22.33±0.88	B 2.20±0.06
	10 %	3	AB 24.67±0.33	A 50.33±0.33	A 2.00±0.58	A 23.00±0.58	B 2.23±0.12
	Control	3	B 23.33±0.33	C 45.00±0.58	C 1.33±0.33	C 20.33±0.33	C 1.73±0.09
2nd Week	2.5 %	3	A 25.67±0.88	A 47.00±0.58	C 0.67±0.33	B 21.33±0.33	B 2.07±0.03
	5 %	3	A 25.33±0.33	A 47.67±0.88	B 1.33±0.33	B 22.33±0.88	A 2.30±0.10
	10 %	3	A 25.00±0.58	A 47.33±2.03	A 1.67±0.33	A 23.33±0.33	A 2.30±0.10
	Control	3	B 23.00±0.58	B 45.33±0.33	D 0.67±0.33	C 21.33±0.33	C 1.73±0.03
3rd Week	2.5 %	3	C 24.33±0.33	C 46.67±0.33	C 1.33±0.33	C 20.67±0.33	C 2.07±0.12
	5 %	3	AB 26.33±0.33	B 48.33±0.67	B 1.67±0.88	B 21.67±0.33	B 2.23±0.09
	10 %	3	A 27.67±0.33	A 50.00±0.58	A 2.67±0.33	A 24.33±0.33	A 2.47±0.09
	Control	3	B 25.67±0.33	D 46.00±0.58	B 1.67±0.33	C 20.67±0.33	C 2.00±0.06
4th Week	2.5 %	3	B 24.00±1.15	C 45.33±0.33	B 1.67±0.33	C 21.00±1.73	B 2.23±0.09
	5 %	3	A 26.67±0.33	B 47.33±0.33	A 2.00±0.58	C 21.00±0.58	A 2.30±0.06
	10 %	3	D 22.33±0.88	A 49.67±0.33	A 2.00±0.58	A 23.00±0.58	A 2.37±0.03
	Control	3	C 23.67±0.33	D 46.67±0.33	C 1.33±0.33	B 22.33±0.88	C 2.03±0.09

Table (5): Means ± S. E of Albumin, globulin, Total protein and Albumin/ globulin ratio among different treatments at different weeks.

Week	Treatment	N	Albumin	Globulin	Total protein	A/g ratio
			Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error
1st Week	2.5 %	3	C 2.30±0.06	A 1.87±0.09	B 4.17±0.03	D 1.24±0.09
	5 %	3	A 2.83±0.15	B 1.53±0.03	A 4.37±0.15	A 1.85±0.11
	10 %	3	B 2.40±0.17	C 1.40±0.15	D 3.80±0.21	B 1.76±0.25
	Control	3	D 2.20±0.06	D 1.33±0.03	C 3.53±0.09	C 1.65±0.02
2nd Week	2.5 %	3	C 2.20±0.06	A 2.30±0.06	B 4.50±0.12	C 0.96±0.00
	5 %	3	A 3.27±0.03	D 1.33±0.09	A 4.60±0.06	A 2.47±0.18
	10 %	3	B 3.17±0.03	C 1.37±0.20	B 4.53±0.18	A 2.44±0.40
	Control	3	C 2.13±0.03	B 1.43±0.07	C 3.57±0.03	B 1.50±0.10
3rd Week	2.5 %	3	C 2.17±0.03	A 2.20±0.10	B 4.37±0.12	C 0.99±0.04
	5 %	3	A 3.13±0.03	C 1.47±0.09	A 4.60±0.06	A 2.16±0.16
	10 %	3	B 3.00±0.06	D 1.37±0.09	B 4.37±0.15	A 2.21±0.11
	Control	3	D 2.07±0.12	B 1.70±0.15	C 3.77±0.03	B 1.25±0.20
4th Week	2.5 %	3	B 2.30±0.06	A 1.83±0.03	B 4.13±0.03	C 1.26±0.05
	5 %	3	A 2.87±0.18	B 1.67±0.19	A 4.53±0.09	A 1.79±0.33
	10 %	3	B 2.33±0.03	A 1.87±0.03	B 4.20±.06	C 1.25±0.02
	Control	3	C 2.20±0.06	C 1.43±0.03	C 3.63±0.03	B 1.54±0.07
	Total	48	2.54±0.06	1.63±0.05	4.17±0.06	1.64±0.08

For each week: Means within the same column carrying different letters are significantly different at ($P<0.01$).

Table (6): Antibody titers (Log2) in different treated groups.

Groups	Week Post-vaccination			
	1st Week	2nd Week	3rd Week	4th Week
Control	Cc 2±0.1	Cb 3±0.1	Ca 4±0.3	Da 4±0.3
2.5 % Spirulina	Bb 5±0.3	Ba 6±0.2	Aa 6±0.5	Cb 5±0.4
5% Spirulina	Aa 7±0.4	Aa 7±0.5	Bc 5±0.3	Bb 6±0.5
10 % Spirulina	Aa 7±0.4	Bb 6±0.3	Ab 6±0.3	Aa 7±0.4

-Capital letters indicated that: Means within the same column of different letters are significantly different at ($P < 0.01$).

-Small letters indicated that: Means within the same raw of different letters are significantly different at ($P < 0.01$).

Table (7): Mortality number and relative level of protection of ***O. niloticus*** among different treatments.

Treatment	Mortality number	RLP %
Vaccinated fish fed on diet + 2.5% Spirulina	Zero/10	100
Non vaccinated fish fed on diet + 2.5% Spirulina	4/10	60
Vaccinated fish fed on diet + 5% Spirulina	1/10	90
Non vaccinated fish fed on diet +5% Spirulina	6/10	40
Vaccinated fish fed on diet +10% Spirulina	1/10	90
Non vaccinated fish fed on diet +10% Spirulina	5/10	50
Vaccinated fish fed on diet + Zero % Spirulina (Basal diet)	6/10	40
Non vaccinated fish fed on diet + Zero % Spirulina (Basal diet)	10/10	Zero

RLP = 1 – (Mortality in vaccinated fish/Mortality in control fish) x 100.

DISCUSSION

It remains to be seen if the use of benefit algae "Spirulina" can offer protection against the many pathogens which are plaguing the aquaculture industry. The use of antibiotic drugs to control these pathogens is ineffective and has undesirable safety consequences for consumers; Supplementation with Spirulina of the feed of these aquacultural animals may offer a better alternative.In order to overcome the diseases incidence in tilapia, Spirulina Supplementation is being studied ***(Watanuki et al., 2006).***

In the present work, we spot light on immunostimulatory effects of Spirulina on ***O. niloticus*** which is about 95% of cultured fish in Egypt where the fish were fed on Spirulina supplemented diet for 8 weeks. The effects of Spirulina on serum proteins, differential leucocytic count (Lymphocytes and monocytes,) as well as phagocytic activity, index and the level of antibody titer, relative level of protection, were determined.

The present investigation revealed elevation of all parameters of immune response of ***O. niloticus*** as non specific types as (WBCs, Differential leucocytic count, phagocytic activity) and specific types as (antibody titer and relative level of protection).

The blood parameters as leucocytic counts and differential leucocytic count have diagnostic importance and usually readily respond to identical factors such as physical, chemical and biological stressors ***(Soliman, 1996).***

The results indicated that, the WBCs values had the highest values during addition of Spirulina, also, there was increasing in lymphocytes and monocytes in groups fed on Spirulina than the control groups. These results are in agreement to data obtained by **(*Edvington et al., 1994)*** who cleared that fish not received any immunostimulants or live under stress conditions showed decrease leucocytes counts and increase susceptibility to infection. Moreover, ***Abdel-Tawwab et al. (2008)*** stated fish fed on diets containing 2.5 - 10.0 g Spirulina / kg diet exhibited similar WBCs counts while the low counts of WBCs were obtained at the control diet. While no significant changes in lymphocytes were observed at 2.5 – 10.0 g Spirulina / kg diet ($P < 0.05$).

But our results are contrary to data obtained by ***Phromkunthong and Pipattanawattanakul (2005)*** who mentioned that the supplementation of Spirulina sp. in hybrid catfish, ***Clarias macrocephalus x Clarias gariepinus*** resulted in no changes of blood parameters or histology.

Our results may be due to there is evidence that c-phycocyanin and polysaccharides of Spirulina enhance white blood cell production ***(Qureshi et al., 1996).*** Studies have shown that phycocyanin affects the stem cells which found in the bone marrow. Stem cells are the "grandmother" of both the white blood cells that make up the cellular immune system and the red blood cells that oxygenate the body ***(Kithja, 2005).***

Several researches indicated that ***S. platensis*** has immuno-enhancing properties in both animals and humans, but to date, there is limited

information concerning the immunostimulatory effects of Spirulina in fish. Some recent studies have shown that feeding Spirulina to fish and poultry results in increased disease resistance and in improved survival and growth rates which may be attributed to an improvement of immune functions ***(Hayashi et al., 1998).***

The results indicated that, fish groups supplemented with Spirulina showed increased of phagocytic activity and phagocytic index until the 4th weeks from feeding Spirulina supplemented diets. This data is in agreement with data obtained by ***Liu et al. (1991)*** who reported that intraperitoneally injected polysaccharides of a hot-water extract of Spirulina increased the percentage of peritoneal phagocytic cells besides increasing the hemolysin contents in the blood of mice. Our results are also parallel to data obtained by ***Qureshi and Ali (1996)*** who showed that the percentage of phagocytic macrophages was increased when cats were administered water-soluble extract of ***S. platensis***, also, increased phagocytic activity was also observed in other animals such as mice and chicken. Also, ***Watanuki et al. (2006)*** cleared that dietary inclusion of ***S. platensis*** for carp, ***Cyprinus carpio*** showed enhanced responses of phagocytic activity and super oxide anion production in kidney phagocytic cells. This activation of kidney cells was observed for at least 5 days post treatment while similar data obtained by ***Khalil et al. (2007)*** who cleared that Spirulina enhance the phagocytic activity and phagocytic index of ***O. niloticus***.

Our results may be attributed to the fact that Spirulina augmented the expression of *cytokine genes* in the head kidney leucocytes of fish. Cytokines are simple polypeptides or glycoprotein that acts as signaling molecules within the immune system ***(Thomson, 1994).*** Moreover, Oral administration of Spirulina to carp leads to augmented the expression of IL-1*β* and TNF-*α* genes in the kidney leucocytes, thus, dietary spirulina can be added to the list of substances that stimulate the expression of these important cytokines in fish ***(Watanuki et al., 2006)*.**

The total serum proteins were useful in diagnosis of fish diseases ***(Mulcathy, 1967).*** In the present work, the significant increase in albumin, globulin and total protein and increase of albumin/globulin (A/G) ratio in the groups fed on Spirulina than control group. These results were parallel to that obtained by ***El-Kafoury (2006)*** who mentioned that ***O.niloticus*** receives immunostimulant showed increase in albumin, globulin and total protein.

These results may be due to the effect of Spirulina in fish causes regeneration of the liver cells and increasing the immune status of fish body with increasing of serum proteins ***(Manning and Wyatte, 1984 a, b).***

Antibody response is known to be an important competent of the fish immune system and the ability to monitor such a response is essential for to understanding adaptive immunity. Passive immunization studies have shown that injection of high titer anti-pathogen serum into fish before or soon after pathogen challenge can confer significant protection

(Jian et al., 2005).

In this study the results indicated that, the antibody titers in all Spirulina supplemented groups were higher than the control feed on basal diet where ranged between 2 ± 0.1, 3 ± 0.1, 4 ± 0.3 and 4 ± 0.3 during 1st, 2nd, 3rd and 4th weeks respectively and at the end of the 4th week, the antibody titers ranked in groups where the 10% Spirulina supplemented group come in the first rank, followed by 5% Spirulina supplemented group, then 2.5% Spirulina supplemented group while the control group came in the last rank in values. These results can be explained that the Spirulina may contain some factors that enhancing the activity of the fish and stimulating the antibody secretion leads to increasing the antibody titers. Challenge experiment confirmed that antibody titers were correlated with protection from ***A. hydrophila*** challenge, where Spirulina - treated tilapia and vaccinated groups conferred good protection against ***A. hydrophila*** in the challenge test in comparison to the basal diet fed group.

These results are parallel to data obtained by ***Phromkunthong and Pipattanawattanakul (2005)*** who cleared that feeds containing varying percentages of dry Spirulina help in development of antibody levels against bacteria ***Aeromonas hydrophila*** in hybrid catfish, ***Clarias macrocephalus x Clarias gariepinus*****.** Moreover, ***Khalil et al. (2007)*** who cleared that the fish fed with Spirulina and vaccinated fish conferred protection against ***Aeromonas hydrophila*** challenge 80% and 70% relative percentage survival (RPS), respectively.

These results may be attributed to the fact that antibody produced due to the immuno-stimulatory effects of Spirulina and vaccine plays a role in conferring significant protection. Thus, this results show the increased resistance to ***A. hydrophila*** infection on tilapia treated with Spirulina ***Watanuki et al. (2006).*** Also, ***Gekle et al. (1998)*** reported that immunostimulant increased antibody titer in fish exposed to different environmental condition higher than fish without immunostimulant. The immunostimulant caused increasing of serum protein and subsequently immunoglobulin formation. Moreover, the increasing of RLP was proved by higher titer of antibody in case of groups received immunostimulants.

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