

Clinicopathological studies on the effect of spirulina in culture Nile tilapia.

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Abstract

This study was undertaken to study the effect of spirulina (*Arthrospir platensis*) on some serum biochemical parameters of *O.niloticus*. A total of 270 fish (50±5 g) were randomly distributed into six groups each at a rate of 15 fish per aquarium and fed on a diet containing 0.0, 5.0 or 10.0 g spirulina/kg diet for 6 weeks. Each subdivided into three equal replicates. After the feeding trial, fish of each treatment were challenged by pathogenic *Pseudomonas. fluorescens* which was given by I/P injection. The blood samples were taken after 4 and 6 weeks for serum biochemical examinations. The results showed that spirulina improved serum AST, ALT, total protein, albumin, globulin, glucose, creatinine, uric acid and cholesterol in groups supplemented with spirulina. Moreover, spirulina enhanced histopathological lesions of fish infected with *P. fluorescens*. These results indicate that spirulina supplementation is promising for disease prevention in tilapia culture, and the optimum level of spirulina in fish diet is 10.0 g per kg diet.

Introduction:

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*).

Spirulina (*Spirulina platensis*) is a freshwater blue-green filamentous alga, and it is receiving increasing attention for its bioactive

components such as vitamins, protein (60–70%), minerals, polyunsaturated fatty acids, carotenes and other pigments that have antioxidants activity (*Madhava et al, 2000; Lin et al, 2007*). Moreover, *Bermejo et al (2008)* reported that most antioxidant capacities of Spirulina protean extract are attributable to the biliproteins contained in this microalga, such as phycocyanin. The previous authors suggested that spirulina could be used to produce a natural dietary antioxidant supplement or added to healthy

food products, such as cereals, fruit bars or drinks, to prevent some chronic diseases where free radicals are involved. Also, *El-Kafoury (2006)* mentioned that *Oreochromis niloticus* received immunostimulant showed increase in albumen, globulin and total protein. In this regards, *Abdel-Tawwab et al, (2008)* sated that Serum glucose, lipids, and protein, albumin, and globulin values of *Oreochromis niloticus* increased with the increase of *Spirulina* supplementation. Also in the same line with *Kaoud et al, (2012)* who added that the addition of dried *Spirulina platensis* to *Oreochromis niloticus* exposed to Hg improves the ALT and AST activity to be nearly as in the control. This study was conducted to determine the effects of graded levels of spirulina (*A. plantensis*) on some serum biochemical parameters and histopathological findings of *Oreochromis niloticus*.

Materials and Methods:

Fish: A total number of 270 *O niloticus* with an average body weight of 50 ± 5 g were obtained from fish hatchery at the Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. They were transported in sterile plastic bags containing water enriched by oxygen (2/3) to the lab of the Dept. of Fish Diseases, Faculty of Veterinary Medicine, Suez Canal University. They were kept for two weeks under observation for

acclimatization in glass aquaria (100×40×50cm). Fish were fed on the control diet for 2 weeks. The water was removed daily.

Aquaria: Fish were randomly distributed into six groups at a rate of 15 fish per aquaria. These aquaria were used for holding the experimental fish throughout the period of the present study, (triplicate each treatment). Each aquarium was supplied with chlorine free tap water (*Innes, 1966*). The water temperature was kept at 22 ± 1 °C. The continuous aeration was maintained in each aquarium using an electric air pumping compressors.

Diet preparation: A basal diet was formulated to contain 30.6% crude protein diet table (1). The diet was daily provided at a fixed feeding ratio of 3% of body weight of fish according to *Eurell et al (1979)*. The daily amount of food was offered as two equal meals /day on two occasions over the day (9Am and 12 PM).

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

Blood sampling: At the 4 weeks and after 6 weeks, fish were fasted for 24 hours immediately prior to blood sampling and five fish per aquaria were randomly chosen. The blood was extracted from the caudal blood vessels. Blood was collected in eppendorf tubes with no anticoagulant in order to clot at 4°C

and centrifuged at 5000 rpm for 5 min at room temperature.

Serum biochemical examination: Glucose was determined calorimetrically according to *Trinder (1969)*. Total protein content in serum was determined calorimetrically according to *Henry (1964)*. Albumin and globulin in plasma were determined calorimetrically according to *Drupt (1974)*. The serum level of AST and ALT was determined colorimetrically according to *Reitman and Frankel (1957)* serum creatinine level (mg/dl) was estimated according to *Henry et al (1974)* serum uric acid (mg/d) was determined according to the method of *Caraway (1963)* and serum cholesterol (mg/d) was determined according to the method of *Joseph et al (1972)*.

Challenge test: After month, fish from each treatment groups (10fish/

aquarium). Were challenged with pathogenic *Pseudomonas fluorescens*. The fish in first group were injected intraperitoneally with 0.2 ml sterile saline containing (1.5×10^8 / ml) pathogenic strain, according to *El-Attar and Moustafa (1996)*.

Histopathological examination: tissue specimen from liver and kidney were examined according to *(Drury and Willington, 1980)*.

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

Table 1: Design of the experiment of feeding of spirulina platensis to tilapia (*O. niloticus*):

Treatments (Groups)	Diet	Infection
I	Basal diet	Not infected
II	Basal diet containing 5g <i>Spirulina</i> /kg diet	Not infected
III	Basal diet containing 10g <i>Spirulina</i> /kg diet	Not infected
IV	Basal diet	Infected
V	Basal diet containing 5g <i>Spirulina</i> /kg diet	Infected
VI	Basal diet containing 10g <i>Spirulina</i> /kg diet	Infected

Results

Serum biochemical changes:

The results after 4 weeks showed that both transaminases enzymes (ALT and AST) were significantly decreased with the increase of spirulina supplementation compared to control. The results after 6 weeks showed that serum ALT and AST were stable in fish fed on spirulina while these enzymes were significantly increased in control group that fed on basal diet and infected with *P. fluorescens* group (IV).

The present study showed that fish fed on diets containing 10.0 g spirulina/kg group (III) diet exhibited higher total serum protein, albumin, and globulin as compared with fish fed the control diet (I). On the other hand the protein, albumin and globulin values were significant decreased in control group that fed on basal diet and infected with *P. fluorescens*, (group IV) while significant increased values were obtained in infected fish fed on spirulina (groups V and VI) compared to control infected (group IV).

The serum glucose level was significantly increased in fish with the increase of spirulina supplementation and the highest values were obtained at 1% spirulina group (group III) after 4 weeks. While, serum glucose levels were significantly increased in infected groups in which highest values obtained in 1% spirulina infected group (VI), the lowest

values obtained in control group (IV) after 6 weeks.

The concentration of serum uric acid (mg/dl) and creatinine (mg/dl) of *O. niloticus* after 4 weeks were decreased by addition of spirulina in diet while, after 6 weeks showed that serum creatinine levels in non infected groups were decreased. Serum uric acid levels were stable in fish fed on spirulina. While the infected groups showed that the highest values of were obtained in control infected group (IV) and decreased by the increase of spirulina supplementation in diet.

After 4 weeks, serum cholesterol was significantly decreased in fish with the increase of spirulina supplementation. After 6 weeks serum cholesterol was increased in infected groups, the highest values in fish fed on basal diet infected with *P. fluorescens* (group IV), while cholesterol levels return to normal level by increase of spirulina supplementation in diet (table 2).

Histopathological results:

Histopathological results showed that hepatic melano-macrophage centers and Kupffer cells appeared more activated. Also, kidney and spleen showed activations of melano-macrophage centers in case of fish groups fed on spirulina supplemented feed than fish groups fed on basal diet.

However, the hepatic tissue of infected fish with *P. fluorescens* showed, In case of Group (IV): their liver showed congestion of

hepatoportals vein and hepatic sinusoid, hyperplasia of epithelial lining of bile duct, inactivation of pancreatic acini which infiltrated by leukocytes and vacuolar degeneration of the hepatic parenchyma (figure 2). Group (V): hepatocytes showed mild swelling, hyperplasia of melanomacrophage center and vacuolation of hepatocytes (figure 3). Group (VI): fish fed on basal diet and 1% spirulina and infected with pseudomonas fluorescence, their liver showed mild to moderate degree of degeneration (figure 4). Group IV, showed severe hydropic degeneration of the glomerular and

renal tubular epithelium, variable degree of depletion of the hemopoietic elements, congestion and focal hemorrhages of the peritubular blood vessels (figure 6). Group V, showed mild congestion of some peritubular blood vessels, hyperplasia of melanomacrophage center and Hydropic degenerated of some renal tubules (figure 7). Group VI, showed mild, focal degeneration of some tubular epithelium. The renal melanomacrophage centers were variably enlarged and hyper-activated and focal proliferation of the interstitial lymphoid elements also detected (figure 8).

Table (2) Serum biochemical changes of *O. nloticus* fed on practical diets containing different levels of spirulina after 4 weeks:

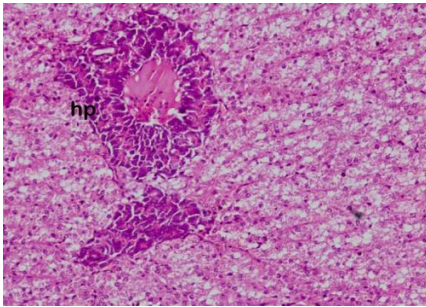
Items	Control (I)	Spirulina 0.5% (II)	Spirulina 1% (III)	Control infected (IV)	Spirulina 0.5% infected(V)	Spirulina 1% infected (VI)
ALT (I.U./L)	13 ±0.20 ^a	12.7±0.15 ^a	7±0.23 ^b	13.2±0.69 ^a	12.8±0.15 ^a	6.9±0.51 ^b
AST (I.U./L)	136±0.70 ^a	125±0.29 ^b	117±0.12 ^c	135.6±0.52 ^a	125.5±0.78 ^b	117.03±0.26 ^c
Total protein (mg/dl)	3.4±0.35 ^b	3.6±0.26 ^{ab}	4.2±0.12 ^a	3.3±0.20 ^b	3.7±0.69 ^{ab}	4.1±0.69 ^a
Albumin (mg/dl)	2.1±0.30 ^b	2.2±0.12 ^b	2.73±0.88 ^a	2.1±0.06 ^b	2.23±0.15 ^b	2.6±0.34 ^a
Globulin (mg/dl)	1.35±0.05 ^a	1.4±0.28 ^a	1.5±0.03 ^a	1.33±0.03 ^a	1.44±0.28 ^a	1.86±0.21 ^a
Glucose (mg/dl)	53.1±0.10 ^c	60±0.17 ^b	65±0.40 ^a	53.07±0.02 ^c	61.1±0.72 ^{ab}	65.4±0.42 ^a
Creatinine (mg/dl)	0.39±0.01 ^a	0.2±0.02 ^b	0.14±0.03 ^c	0.38±0.05 ^a	0.21±0.02 ^b	0.15±0.04 ^c
Uric acid (mg/dl)	0.5±0.04 ^a	0.30±0.01 ^b	0.11±0.01 ^c	0.49±0.03 ^a	0.32±0.02 ^b	0.16±0.05 ^c
Cholesterol (mg/dl)	166±0.30 ^a	114±0.23 ^b	107±0.46 ^c	166.2±0.36 ^a	114.3±0.26 ^b	106.9±0.52 ^c

Data in the same row with different superscript are significantly different ($P < 0.05$)

Table (3) Serum biochemical changes of *O. niloticus* fed on practical diets containing different levels of spirulina after 6weeks:

Items	Control (I)	Spirulina 0.5% (II)	Spirulina 1% (III)	Control infected (IV)	Spirulina 0.5% infected (V)	Spirulina 1% infected (VI)
ALT (I.U./L)	12.9±0.54 ^d	7±0.12 ^e	6±0.40 ^e	49±0.12 ^a	25±0.23 ^b	16±0.46 ^c
AST (I.U./L)	135±0.05 ^d	118±0.17 ^e	105±0.35 ^f	235 ±0.23 ^a	201±0.36 ^b	181.3±0.44 ^c
Total protein (mg/dl)	3.17±0.19 ^c	3.66±0.29 ^{bc}	5±0.29 ^a	2.9 ±0.26 ^c	3.3±0.12 ^{bc}	3.93±0.21 ^b
Albumin (mg/dl)	2.2±0.12 ^{abc}	2.7±0.12 ^{ab}	2.8±0.05 ^a	1.7±0.11 ^c	2±0.29 ^{bc}	2.17±0.39 ^{abc}
Globulin (mg/dl)	0.96±0.49 ^b	0.97±0.68 ^b	2.3±0.45 ^a	1.26±0.37 ^{ab}	1.33±0.66 ^{ab}	1.55±0.21 ^{ab}
Glucose (mg/dl)	65±0.60 ^f	82±0.30 ^e	106±0.70 ^b	96±0.40 ^d	100±0.70 ^c	112±0.30 ^a
Creatinine (mg/dl)	0.35±0.03 ^b	0.19±0.02 ^c	0.13±0.02 ^c	0.46±0.04 ^a	0.19±0.02 ^c	0.12±0.03 ^c
Uric acid (mg/dl)	0.50±0.05 ^c	0.18±0.02 ^d	0.24±0.03 ^d	1±0.06 ^a	0.80±0.05 ^b	0.80±0.06 ^b
Cholesterol (mg/dl)	150.6±0.40 ^b	132±0.23 ^d	137±0.12 ^c	173±0.23 ^a	150.9±0.32 ^b	150.6±0.72 ^b

Data in the same raw with different superscript are significantly different ($P < 0.05$)



Figure(1): liver of Tilapia fish, group control showing normal structure of hepatic cells and hepatopancreas (hp). H&E. X 400.

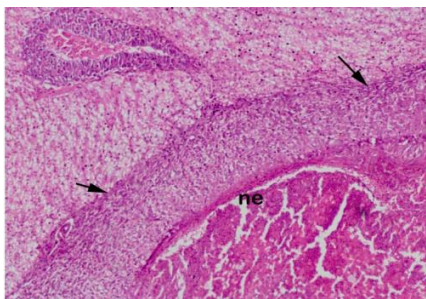


Figure (2): liver of Tilapia fish, group IV showing group massive necrosis of hepatic tissue (ne) & mononuclear cell infiltration (arrows). H&E. X 200.

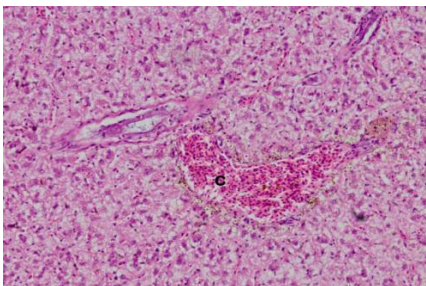


Figure (3): liver of Tilapia fish, group V, showing mild congestion of blood vessels (c). H&E. X 200.

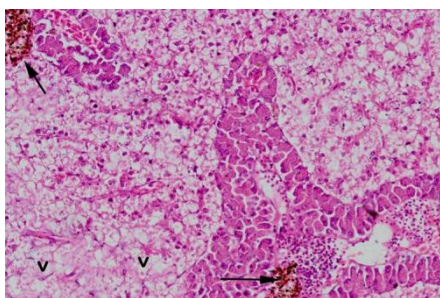


Figure (4): liver of Tilapia fish, group VI showing mild vacuolation (v) and activation of melanomacrophage centers (arrows). H&E. X 200.

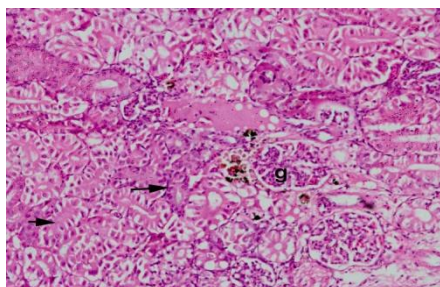


Figure (5): kidney of Tilapia fish, group I showing normal glomeruli (g) and renal tubules (arrows). H&E. X 200.

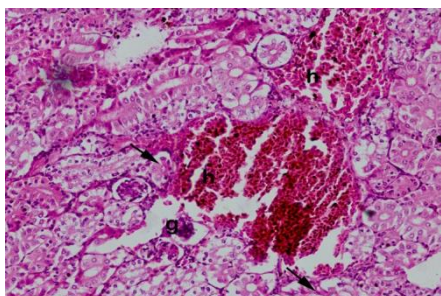


Figure (6): kidney of Tilapia fish, group IV showing hemorrhage(h), degeneration and necrosis of renal tubules (arrows).

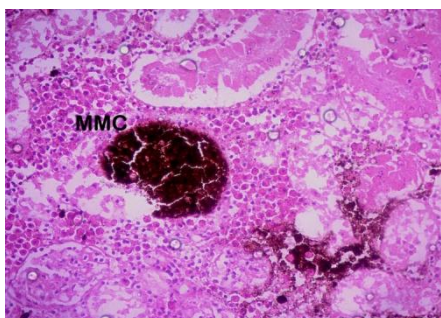


Figure (7): kidney of Tilapia fish, group V showing mild degeneration, activation of lymphoid tissue and activation of melanomacrophage centers (MMC). H&E. X 200.

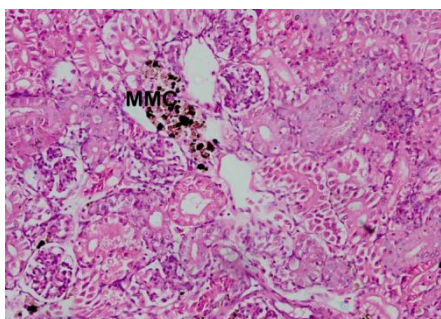


Figure (8): kidney of Tilapia fish, group VI showing activation of lymphoid tissue and melanomacrophage centers (MMC) . H&E. X 200

Discussion

The activity of AST and ALT enzymes in blood may also be used as a stress indicator the significant changes in the activities of these enzymes in blood plasma indicate tissue impairment caused by stress (Svoboda, 2001). In the present study, enzymes (ALT and AST) were significantly increased in control group. This result agreed with Belal *et al* (2012), who revealed that fish fed with spirulina supplementation significantly decreased (AST) and (ALT) values compared with control. Also, in this concern, Ragap (2009) mentioned that the liver function enzymes serum S.GOT and S. GPT were stable in fish fed on spirulina while these enzymes were significantly increased in control group that feed in basal diet. The obtained results were proved by histological results where the hepatic melanomacrophage centers and Kupffer cells appeared more activated incase of fish groups fed on spirulina supplemented feed than fish groups fed on basal diet.

Also, the results after 6 weeks showed that serum ALT and AST were stable in fish fed on spirulina while these enzymes were significantly increased in control groups. This agreed with Kaoud *et al*, 2012 who added that the addition of dried *Spirulina platensis* to *Oreochromis niloticus* exposed to Hg improves the ALT and AST activity to be nearly as in the control. In this regard, Wafaa

(2007) who revealed that increase ALT, AST liver enzymes in the experimentally infected fish with *Pseudomonas fluorescens*. The elevation in the ALT and AST attributed to the damage of hepatic cells and others tissues and escaping of these enzymes to the blood (Coles, 1986) and/or the increase in concentration of AST and ALT in blood plasma indicates impairment of parenchymatous organs mainly liver. In addition, the increase of plasma AST and ALT may be attributed to the hepatocellular damage or cellular degradation in liver, spleen or muscles (Yamawaki *et al*, 1986 and Kaoud *et al*, 2012). So indicating that spirulina has to some extent a protective role induced tissue injuries. The exact cause of this protective role in recovering tissue damages is not fully understood (Islam *et al*, 2009). Histopathological findings indicated that there were congestion of hepatoportal vein and hepatic sinusoid, hyperplasia of epithelial lining of bile duct. Inactivation of pancreatic acini which infiltrated by leukococysts and vacuolar degeneration of the hepatic parenchyma of fish group fed on normal diet and infected with *pseudomonas fluorescence* (control +v) While, groups V and VI showed regeneration (mild to moderate regenerations) with hyperactivity of melenomacrophage centers of haemopiotic organs. This results may be due to the effect of extracellular proteases produced by

P. fluorescens which attack endothelial lining of blood vessels and parenchymatous organ causing the haemorrhagic phenomena as well as the degenerative changes (**Ehab, 1991**).

In the present study, the results showed that fish fed on diets containing 10.0 g Spirulina/kg group (III) diet exhibited higher total protein, albumin, and globulin as compared with fish fed the control diet (I). These results were parallel to that obtained by **Abdel-Tawwab et al (2008)**, **Ragap(2009)**, **Andrews et al (2011)** and **Belal et al (2012)** who revealed that the serum total protein, albumin, and globulin values were increased by addition of spirulina in diet. Commonly, increases in the levels of plasma total protein, albumin and globulin in fish are thought to be associated with a stronger innate immune response (**Wiegertjes et al, 1996**). On the other hand the protein, albumin and globulin values were significant decreased in control group that fed on basal diet and infected with *P. fluorescens*, (group IV) while significant increased values in infected fish fed on spirulina(groups V and VI). In this regards, **Sivagurunathan et al (2012)** recorded that significant increase was observed in serum total protein and albumin levels of *Cirrhinus mrigala* infected with *P. fluorescens* fed on Lotus for 40 days. The increase in serum protein content might be in part due to an increase in the WBC, which is a

major source of serum protein production such as lysozyme, complement factors and bactericidal peptides (**Misra et al, 2006**).

The blood glucose level is a sensitive indicator in fish for environmental stress (**Silbergeld, 1974**). The result of this study after 4 weeks showed that the glucose level was significantly increased in fish with the increase of spirulina supplementation. These results were parallel to that obtained by **Abdel-Tawwab et al (2008)** and **Belal et al (2012)**, that fish fed with diets containing 1% spirulina exhibited higher glucose levels. These results may be attributed to its bioactive components such as 13.5% carbohydrates; the sugar composition is mainly composed of glucose, along with rhamnose, mannose, xylose, galactose, and two unusual sugars: 2-O-mehtyl-1-rhamnose and 3-Omethyl-1-rhamnose. (**Madhava et al, 2000**). Also, after 6 weeks showed that serum glucose levels were significantly increased in infected groups. The elevation in glucose level due to stress and stimulation of glycogenolysis (**Soivio et al, 1974**). And/or may be due to mobilization of glucose in response to stress is generally accepted as a mean of providing extra energy resources enabling the fish to overcome the disturbance (**Arends et al, 1999**).

The concentration of uric acid (mg/dl) and serum creatinine (mg/dl) of *O. niloticus* after 4 weeks

were highest in control group fed on basal diet (I). This result clearly shows the protective effect of spirulina that may be due to C-phycocyanin which is one of the major constituent of spirulina protect renal cell injury (**Farooq *et al*, 2004**). After 6 weeks the infected groups showed that the highest values were obtained in control infected group (IV) and decreased by the increase of spirulina). The elevation in serum creatinine and serum uric acid levels may be due to alteration in normal physiology of kidney due to bacterial toxin. (**Coles, 1986**).

After 4 weeks serum cholesterol was significantly decreased in fish with the increase of spirulina. While, after 6 weeks serum cholesterol was increased in infected groups, in which the highest values were obtained in control infected group. These results agreed with **Heidarpour *et al* (2011)** who revealed that the fish (*Holstein calves*) showed significant reduction in plasma cholesterol by increasing spirulina in diet. This cholesterol serum reduction has been stated as the effect of spirulina on lipoproteins metabolism and the increase of the lipoprotein enzyme activity levels (**Karkos *et al*, 2008**).

The obtained results were proved by histological results where hydropic degeneration of the glomerular and renal tubular epithelium, variable degree of depletion of the hemopiotic elements, congestion and focal hemorrhages of the

peritubular blood vessels of fish group fed on normal diet and infected with *pseudomonas fluorescens* (control +v) While, groups V and VI showed regeneration (mild to moderate regenerations) with hyperactivity of melanomacrophage centers of haemopiotic organs.

The present study concluded that Spirulina positively improved serum biochemical parameters 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.

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الملخص العربي

بعض الدراسات الباثولوجيا الإكلينيكية على تأثير السبيرولينا في سمك البلطي النيلي
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البحار

ما زال استخدام الطحالب الدقيقة في مجال تغذية الأسماك يتم على نطاق ضيق. وقد أجريت هذه الدراسة بهدف دراسة التأثيرات الناتجة عن إضافة طحلب السبيرولينا (السبيرولينا بلاتنيسيس) لأعلاف أسماك البلطي النيلي بمستويات مختلفة من مسحوق الطحالب. وقد استخدم في هذه الدراسة 270 سمكة بلطي نيلي تم تجميعه من مزرعة مركز بحوث الأسماك بالعباسة بأبوحمد شرقية وكان متوسط وزن السمكة الواحدة 50 جرام تقريبا وقد أحضرت الأسماك إلى المعمل بقسم أمراض ورعاية الأسماك بكلية الطب البيطري جامعة قناة السويس في أكياس بلاستيكية مزودة بنسبة أكسجين 3. تم استخدام 3 علائق تجريبية بإضافة مسحوق الطحالب في علائق الأسماك المترنة بنسب مختلفة. وقد تم تقسيم الأسماك إلى ست مجموعات بحيث كل مجموعة أسماك مقسمة إلى ثلاث مكررات لكل معاملة لكل معاملة يحتوى كل مكرر على عدد 15 سمكة في أحواض زجاجية وذلك لمدة ستة اسابيع وتركت هذه الأسماك للأقلمة لمدة أسبوعين قبل بداية التجربة.

- المجموعة الأولى :- تم تغذيتها على العليقة الأولى والتي تتكون من عليقة أسماك مترنة
- المجموعة الثانية :- تم تغذيتها على العليقة الثانية والتي تتكون من عليقة أسماك مترنة مضاف إليها نسبة 0.5% من مسحوق نفس الطحلب.

- المجموعة الثالثة :- تم تغذيتها على العليقة الثالثة والتي تتكون من عليقة أسماك متزنة مضاف إليها نسبة ١% من مسحوق نفس الطحلب.
- المجموعة الرابعة :- تم تغذيتها على العليقة الأولى والتي تتكون من عليقة أسماك متزنة بدون إضافة مسحوق نفس الطحلب لها وعرضت للعدوى بميكروب السودو موناس فلورسنس في الغشاء البريتوني بجرعة ٢,٠ مل في نهاية التجربة.
- المجموعة الخامسة :- تم تغذيتها على العليقة الثانية والتي تتكون من عليقة أسماك متزنة مضاف إليها نسبة ٥,٠% من مسحوق نفس الطحلب وعرضت للعدوى بنفس الميكروب في نهاية التجربة.
- المجموعة السادسة :- تم تغذيتها على العليقة الثالثة والتي تتكون من عليقة أسماك متزنة مضاف إليها نسبة ١% من مسحوق نفس الطحلب وعرضت للعدوى بنفس الميكروب في نهاية التجربة. وأسفرت هذه الدراسة عن النتائج التالية:أوضحت الدراسة زيادة في نسبة الألبومين&الجلوبولين &البروتين الكلى في مجاميع الأسماك التي تغذيت على عليقه مزودة بمسحوق طحلب السبيرولينا بالمقارنة بالمجموعة الأولى. وقد لوحظ انخفاض في مستوى أنزيمات الكبد في مجموعات الأسماك المغذاه على عليقة أسماك مذودة بمسحوق طحلب السبيرولينا في جميع المعاملات خلال فترة التجربة بالمقارنة بالمجموعة الأولى. بعد العدوى بميكروب السودوموناس لوحظ زيادة بأنزيمات الكبد في المجموعة الأولى مع ثبات الأنزيمات للمعدل الطبيعي في المجموعة الخامسة والسادسة.كما لوحظ أيضا بعد العدوى زيادة في الكرياتينين وحمض اليوريك بالدم في المجموعة الأولى مع ثباتها في المجموعة الخامسة والسادسة. كما كان هناك ارتفاعا واضحا في نسبة الجلوكوز في المجموعة الثالثة و الثانية عن المجموعة الضابطة و بعد العدوى كان هناك ارتفاعا واضحا في نسبة الجلوكوز في جميع المجموعات وجد ايضا انخفاضا ملحوظا في نسبة الكوليسترول في المجموعات المغذاه على السبيرولينا. ومع إمتداد فترة التجربة بالمقارنة بالمجموعة الأولى وجد ان هناك زيادة في نشاط خلايا الميلانومكروفاج الموجودة في الكبد والطحال والكلى بالأسماك المغذاه على العليقة المضاف إليها السبيرولينا. اما بعد العدوى فكانت هناك تغييرات هدامة في خلايا الكبد مع ارتشاح خلوى في المجموعة الرابعة، اما المجموعة السادسة فكان هناك زيادة في نشاط خلايا الميلانومكروفاج.