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THE USE OF *SPIRULINA PLATENSIS* (MICROALGAE) AS FOOD ADDITIVES TO STIMULATE GROWTH AND IMMUNITYMODULATION FOR NILE TILAPIA (*OREOCHROMUS NILOTICUS*) CHALLENGED WITH PATHOGENIC BACTERIA (*PSEUDOMONAS FLUORSCENCE*)

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Abstract

This study was carried out to evaluate the effect of using *Spirulina platensis* as growth and immunity modulator for Nile tilapia *(Oreochromus niloticus*). Fingerlings of *O. niloticus* of *weight (3.3* ± 0.2 g) were randomly distributed into 4 groups, five replicates each group at a rate 20 fish per 100L aquarium and fed by 7 % of their body weight on a diet containing either (0.0, 5, 10,15 gm of *Spirulina platensis* powder/kg basal diet) for 90 days. After the feeding trial, fish of each treatment were challenged by pathogenic bacteria **(***Pseudomonas fluorscence*) which was injected Intra-peritoneal (IP) and they were kept under observation for 14 days to record any abnormal clinical signs and daily mortality rate. Final fish weight, weight gain, and specific growth rate (SGR) which increased significantly ($p<0.05$) with the increase of *Spirulina* addition in fish diets. The highest growth rate was obtained at 15g Spirulina /kg diet and the control diet produced the lowest. The fish groups fed on diet of 15g/kg diet showed the lowest feed conversation ratio (FCR ,1.03) but the control gave the highest FCR (1.45). fish fed *Spirulina* diet produced increased Protein efficiency ratio (PER) with when *Spirulina* additive increased in diet. No regular adverse effects was found in present study neither mortality nor condition factor K ($p<0.05$). The highest values of Hepatosomatic indices (HIS) were significantly recorded in the diet control. Carcass composition of fish fed diets supplemented th *Spirulina platensis* gave higher quality with less fat than

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control and high protein content and ash content. However, the highest red blood cells count (RBCs), haemoglobin content(HB), haematocrite value (Hct), white blood cells count (WBCs) and Nitro-Blue Tetrazolium (NBT) values were obtained at *Spirulina* diet, meanwhile, the lowest values were obtained in control fishes. Also, the total plasma protein and globulin values were increased by the increasing of *Spirulina* additives in diet before and after infection and significantly differences occurred between the treatments than that of the control. Glucose, uric acid, creatinine, Aspartate amino transeferase (AST) and alanine amino transeferase (ALT) were decreased significantly at ($p<0.05$) by increasing the level of *spirulina* in diet after infection. Total fish mortality 14 days after (IP) injection with *Pseudomonas fluorscence* decreased with the increased *Spirulina* level in fish diets. These results indicate that *Spirulina* supplementation is promising for disease prevention in tilapia culture and improved the growth rate and carcass quality.

Key words: *Spirulina*, Nile tilapia, growth performance, feed utilization, haematology,biochemical parameters, NBT, non-specific immunity, *Pseudomonas fluorscence*

INTRODUCTION

Spirulina spp. are highly digestible and don't require special processing, the *Spirulina spp* cells contain 60-65% protein, and are rich with important vitamins such as A and B complex (Borowitzka 1988). Henson (1993) reported that many species of exotic tropical fish and algae constitute an essential part of the diet. *Spirulina spp*. promises five benefits for healthy fish as follows; 1) great profile of natural vitamins and minerals, 2) rich in muco-proteins for healthy skin, 3) providing phycocyanin for better health and reduction of obesity, 4) essential fatty acids for proper organ development, 5) rich in natural color agents such as carotenoids. Addition of *Spirulina spp.* to fish feeds help to solve two problems for growth. Firstly, farmed fish are susceptible to infectious disease. Second, the flavor, texture and skin color are often inferior to the wild fish (Henson 1990). Microalgae are essential in aquaculture feeding, where they are used extensively as live feed. Many investigators started

that microalgae can be used in dried form within production diets for their positive effects on growth and feed utilization, stress and disease resistance (Hirano and Suyama, 1985; Chow and Woo 1990; Liao *et al*., 1990; Cysewski, 1992; Mustafa *et al.*, 1994a & b; Okada *et al.*, 1994; Mustafa *et al*., 1995; Nakagawa *et al*., 2001; Shipton and Britz 2001 and Jun, *et al.*, 2002 &2003). Also, *Spirulina spp.* have been used as a low-level of feed additive to improve the taste, texture or color of the whole fish or (its flesh)..Haematology is gradually becoming routine practice in determining the health fish. Detection of changes in the blood and ability to predict the subsequent health of fish is particularly important when prophylactics management of infectious disease in problematic (Rehulka , 1996) , changes in the blood physiology as well plasma or plasma chemistry values have been shown to associated with infection disease (Pickering, 1986 & Stoskopf, 1993).

Several studies showed that *Spirulina* or its extracts can prevent or inhibit cancers in humans, animals, and fish. Some forms of cancer are the result of damaged cell DNA "out of control", causing uncontrolled cell growth. Cellular biologists have defined a system of special enzymes called Endonuclease which repair damaged DNA to keep cells alive and healthy. When these enzymes are deactivated by oxidation, radiation or toxins, errors in DNA go un-repaired and, cancer may develop. In vitro studies, the unique polysaccharides of *Spirulina* enhance cell nucleus enzyme activity and DNA repair synthesis. Several scientific studies, observing experimental cancers in animals, report high levels of suppression of several important types of cancer (Mathew *et al*., 1995).

Therefore, the present study was carried out to investigate the effect of the used *Spirulina* as growth performance, feed utilization, non-specific immune responses and resistance of Nile tilapia to *Pseudomonas fluorscence* infection and to assess its impact on some physiological parameters of Nile tilapia, *O. niloticus.*

MATERIALS AND METHODS

Isolation and culture of *Spirulina platensis*

The *Spirulina platensis* was isolated from Abbassa water fish ponds in, The World Fish Center, in specific medium. *Spirulina* medium (SAG), is suitable medium for *Spirulina platensis*. Then S. *platensis* cultures were collected from the concrete ponds, washed with tap water, sun dried and grounded into powder according to Richmond (1986).

Fish Culture Management:

Healthy fingerlings of Nile tilapia, *Oreochromis niloticus*, weighing about 3.3 ± 0.2 g/fish were obtained from the nursery ponds of Central Laboratory for Aquaculture Research at Abbassa, Abo-Hammad, Sharkia, Egypt. Fish were acclimated in an indoor tank for 4 weeks under laboratory conditions. The fish were distributed randomly in glass aquaria of 100-liter capacity each, at a rate of 20 fish /aquarium that containing aerated water. Each aquarium was supplied with compressed air via air-stones from air pumps. Well-aerated water supply was provided from a storage fiberglass tank. The temperature was adjusted at 27±1 ^{o}C by means of thermostats. Half of the aquarium's water with fish excreta was siphoned 5day/week and replaced by an equal volume of well-aerated storage water

The fish were assigned to four groups with five replicates. Each of the fish groups fed diets containing different levels of *Spirulina platensis* i.e. 0 (control), 5, 10, and 15 g *Spirulina* /Kg basal diet. Fish were fed frequently a diet containing 25% crude protein (CP) (Table 1) at a rate of 7% of live body weight twice daily for 90 days. Siphoning a portion of water from each aquarium was done every day for waste removal and an equal volume of water was added. Fish in each aquarium was biweekly weighed and subsequently the amount of given feed was calculated. Dead fish were removed and recorded daily.

Proximate Analysis of Diet and Fish:

The basal diet, *Spirulina platensis* powder and samples of 10 fish from each treatment were analyzed using standard methods of the Association of Official Analytical Chemists (AOAC, 1990) for determination of moisture, crude protein, total lipids and ash.

Table 1: Percentage composition of ingredients and proximate compositions of formulated basal diet

Formulated	Basal diet	*Spirulina platensis*
Corn meal	38.7	
Rice bran	25	
Fish meal	20.3	
Starch	7	
Code liver oil	2.5	
Corn oil	1.5	
Vitamins	2	
Minerals	2	
Carboxy-methyl cellulose	1	
Proximate composition		
Moisture	5.2	7.4
Crude protein	24.9	58.5
Crude fat	8.3	6.4
Ash	12.7	9.6
Nitrogen free extract (NFE)	54.1	25.5
Gross energy (Kcal/g)(GE)	4.41	4.297

Growth Parameters:

Growth performance and feed utilization were calculated as following:

Weight gain % = W2 –W1 (g) x 100/ W1

Specific growth rate = 100 (W2 –W1)/ W1 x T

Where W1 and W2 are the initial and final weight, respectively, and T is the number of days in the feeding period.

Feed conversion ratio = FI (B2 + B dead – B1)-1

Where FI, B1 and B2 are the feed intake, the biomass at the start and end, respectively, and B dead is the biomass of the dead fish.

Protein efficiency ratio = (B2 – B1) PI-1

Where B1 and B2 are the biomass at the start and the end of the experiment, and PI is the protein intake.

Bacterial studies:

A bacterial suspension of Pseudomonas fluorescence was diluted with sterile physiological saline to a turbidity equal that of McFarland tube number (7) (the bacterial concentration was 2400 bacteria in millions/ml). A serial ten fold dilutions of this bacterial suspension was prepared as it was stated by Wakabayashi et al., (1981), the initial concentration was 1:1 through 1: 1010.

Challenge test:

After the feeding trial of (non infected fish), 50 fish of each group was IP injected with pathogenic Pseudomonas fluorescence (infected fish by 0.1 ml of 2400 millions cells ml-1) according to Reed and Muench (1938) . All groups were kept under observation for 14 day to record any abnormal clinical signs and the daily mortality rate. The moribund and freshly dead fish were subjected necropsy and bacterial re-isolation.

Hematological Analyses:

At the end of experiment of the feeding trial and challenge test, 5 fish of each aquarium were anaesthetized by Brand of tricaine methanesulfate MS-222 (20 mg l^{-1}) and blood samples were collected from the caudal vein by sterile syringe using EDTA-disodium as an anticoagulant. These blood samples were used for the determination of the erythrocyte and leucocyte counts (Dacie and Lewis, 1984) and hemoglobin content (Van Kampen and Zitstra 1961). Haematocrit value

(Hct) was calculated according to the formulae mentioned by Britton (1963).

Plasma was obtained by centrifugation of the at 3000 rpm for 15 min and nonhaemolyzed plasma was stored in deep freezer at – 20 °C for further biochemical analyses. Glucose was determined, using glucose kits supplied by Boehring Mannheium kit, according to Trinder (1969). Total protein content was determined colorimetrically according to Henry (1964). Albumin and globulin in plasma were determined calorimetrically according to Wotton and Freeman (1982). Activities of aspartate amninotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to Reitman and Frankel (1957).

Uric acid was determined according to Caraway (1957) and also the plasma creatinine was determined according to the colorimetric method of Henry (1964). The production of oxygen radicals by leukocytes was assayed by the reduction of Nitro Blue Tetrazolium (NBT, Sigma-Aldrich Chemical, St .Louis, MO, USA) standard curve of NBT diformazan per milliliter of blood.

Statistical Analysis:

The obtained data were subjected to analysis of variance according to Snedecor and Cochran (1982). Differences between means were at the 5% probability level, using Duncan's new multiple range test (Duncan, 1955).

RESULTS

Growth performance:

Results in table (2) showed that the final weight, weight gain and specific growth rate of *O. niloticus* were significantly increased with the increasing of *Spirulina* level in the diet ($P<0.05$). The highest growth was obtained at 15g *Spirulina* /kg diet, whereas the control diet produced the lowest growth. The highest growth performance in the form of final

weight g/fish, weight gain and % SGR was recorded at 15g of *Spirulina*/Kg diet. No change in fish survival rate which was similar in all treatments (100%) was recorded.

The fish group fed on diet 15g/kg diet had the lowest FCR (1.03) but the control gave the higher FCR (1.45). Fish fed *Spirulina* diet produced increased PER when the Spirulina additive in diet. No regular adverse effects pattern for dietary *Spirulina* was found in the present study neither mortality nor condition factor K. with regard to hepatosomatic indices of fed fish in the experimental diets, the highest values of HSI were significantly recorded in the control group.

Table 2: The mean growth and feed utilization of Nile tilapia *O. niloticus* fed on the experimental diet contain different levels of *Spirulina platensis*.

Items	***Spirulina platensis* levels**			
	Control (0.0%)	**Ttreatment1 0.5%**	**Treatment 2 1.0%**	**Treatment 3 1.5%**
Survival %	100	100	100	100
Initial weight gain(g)	$3.3^{a} \pm 0.4$	$3.3^{a} \pm 0.5$	$3.3^{a} \pm 0.4$	$3.3^{a} \pm 0.4$
Final weight gain (g)	$24.9^{b} \pm 1.32$	$29.7^{a} \pm 1.11$	$30.2^{a} \pm 1.19$	$31.3^{a} \pm 2.12$
Weight gain (%)	654.5	800	815.15	818
SGR(% day)	7.27	8.88	9.05	9.46
FCR	1.45	1.23	1.11	1.03
PER	2.85	3.12	3.42	3.56
HIS	$2.1^{a} \pm 0.23$	$1.92^{b} \pm 0.14$	$1.94^{b} \pm 0.13$	$1.93^{b} \pm 0.15$
K	$1.82^{a} \pm 0.09$	$1.79^{a} \pm 0.068$	$1.8^{a} \pm 0.087$	$1.78^{a} \pm 0.064$

The same letter in the same row is not significantly different at $P < 0.05$

Concerning the proximate chemical analysis of whole fish body, results in table (3) showed that moisture content was approximately similar (72.61-74.5%; P>0.05). Crude protein content in whole fish body was significantly increased with the increasing of *Spirulina* levels (72.2%,73.3% and 72.9% with 0.5%, 1.0% and 1.5% *Spirulina* level,

respectively), and the lowest one was obtained in control (68.8%). On the other hand, content of total lipid showed low values *Spirulina* with (5.2%, 5.6% and 5.4% with 0.5%, 1.0% and 1.5% *Spirulina* level, respectively). Than he control (6.46%). Ash content in whole fish body was significantly increased with diets containing 0.5%, 1.0% and 1.5% *Spirulina* (25.41%, 24.9 and 25.2%, respectively), while the lowest value was observed in the control (23.4%).

Table 3: Proximate chemical analysis (%; on fresh-weight basis) of whole body in O. *niloticus* fed in diet contain different levels of *Spirulina platensis* as additives.

Items	Control (0.0 ‰ Spirulina)	Treatment 1 (5‰ spirulina)	Treatment 2 (10 ‰ Spirulina)	Treatment 3 (15‰Spirulina)
Moisture	72.61^{a}±1.8	73.9^{a} ± 2.3	74.5^{a} ± 1.9	73.6^{a} ± 1.7
Fat	6.46^{a} ±0.6	5.4^{b}± 0.2	5.6^{b} ± 0.7	5.2^{b} ± 0.5
Protein	68.8^{a}±0.95	72.2^{b} ± 1.6	73.3^{b} ± 1.8	72.9^{b} ± 1.5
Ash	23.4^{a} ±1.3	25.41^{b} ± 0.9	24.9^{b} ± 0.54	25.2^{b} ± 1.3

Data are represented as means ± S.E. Means
The same letter in the same row is not significantly different at $P < 0.05$

Haematological parameters:

Erythrocyte counts (RBCS), haemoglobin content (Hb) and haematocrit value (Hct) in the blood of non infected and infected *O. niloticus* were significantly increased by increasing of Spirulina levels in diet when compared with the control groups ($P<0.05$; Table 4), the maximal mean values of erythrocyte count (1.969 ± 0.09 million/ **mm^3**), haemoglobin content (5.62 ± 0.16 g/100ml)and haematocrit value (21.9 ± 1.9 %) was recorded in fish fed with high level of Spirulina. As shown in table (5), no significant variation in the leucocytes count (WBCs), heterophils, lymphocyte and monocyte of nearly all non infected fish under investigation was observed. On the other hand, these values were decreased significantly in all infected fish groups fed on diets containing

5,10, 15 g Spirulina / kg diet when compared with the control fish injected with *Pseudomonas fluorescence.*

Table 4: Changes in erythrocyte (count x 10^6/mm^3), haemoglobin content (g/100ml) and haematocrit value (%) in the blood of Nile tilapia (*O.niloticus* fed in diet contain *Spirulina platensis* additives and with or without injected Pathogenic bacteria *Pseudomonas fluorescence*.

Items	Control (0.0 ‰ Spirulina)	Treatment 1 (5‰ spirulina)	Treatment 2 (10 ‰ Spirulina)	Treatment 3 (15‰Spirulina)
♂RBCS (No.x10^4/mm)				
Non infected	$1.482^a \pm 0.05$	$1.830^b \pm 0.10$	$1.692^a \pm 0.11$	$1.969^b \pm 0.09$
Infected	$1.1^c \pm 0.08$	$1.4^b \pm 0.03$	$1.52^b \pm 0.07$	$1.6^b \pm 0.16$
Haemoglobin Hb (g/100 ml)				
Non infected	$4.1^b \pm 0.24$	$5.2^a \pm 0.2$	$5.49^a \pm 0.05$	$5.62^a \pm 0.16$
Infected	$3.4^c \pm 0.2$	$4.70^a \pm 0.34$	$4.85^a \pm 0.2$	$4.56^a \pm 0.29$
Haematocrit value (Hct) %				
Non infected	$15.2^a \pm 1.23$	$19.5^a \pm 2.1$	$21.3^b \pm 2.3$	$21.9^b \pm 1.9$
Infected	$13.2^a \pm 0.80$	$18.4^b \pm 1.17$	$18.6^b \pm 0.93$	$17.2^b \pm 1.02$

Data are represented as means ± S.E. Means

The same letter in the same row is not significantly different at $P < 0.05$

Table 5: Changes in leucocyte, Heterophils, Lymphocytes Monocytes (No. x 10^3/mm^3) and NBT in the blood of Nile tilapia (*O.niloticus*) fed diet containing different levels of *Spirulina platensis* and with or without infection by Pathogenic bacteria *Pseudomonas fluorescence*.

Items	Control (0.0 ‰ Spirulina)	Treatment 1 (5‰ spirulina)	Treatment 2 (10 ‰ Spirulina)	Treatment 3 (15‰Spirulina)
WBCs (No. x 10^3/mm)				
Non infected	54.33 ^{a} ±2.8	55.78 ^{a} ±3.1	58.8 ^{a} ±3.1	61.9 ^{a} ±2.9
Infected	94.2 ^{a} ± 2.9	73.9 ^{b} ± 3.3	63.4 ^{c} ± 2.8	59.9 ^{c} ± 2.5
Heterophils (No. x 10^3/mm^3)				
Non infected	4.12 ^{a} ±0.21	4.99 ^{a} ±0.3	4.32 ^{a} ±0.18	5.61 ^{a} ±0.45
Infected	8.56 ^{a} ± 0.54	5.39 ^{b} ± 0.33	4.71 ^{b} ± 0.22	3.08 ^{c} ± 0.15
Lymphocytes(No. x 10^3/mm^3)				
Non infected	44.5 ^{a} ± 2.23	44.9 ^{a} ±3.17	48.2 ^{a} ±2.5	47.67 ^{a} ±2.2
Infected	68.1 ^{a} ± 1.9	57.9 ^{b} ± 2.1	51.3 ^{c} ± 0.9	48.3 ^{cd} ± 1.1
Monocytes (No. x 10^3/mm^3)				
Non infected	5.71 ^{a} ±0.9	5.89 ^{a} ±0.96	6.28 ^{a} ±0.76	7.62 ^{a} ±0.47
Infected	17.54 ^{a} ± 1.18	11.63 ^{b} ± 0.71	8.72 ^{c} ± 0.62	5.71 ^{d} ± 0.84
NBT				
Infected	0.324 ^{c} ± 0.02	0.337 ^{b} ± 0.013	0.367 ^{a} ±0.08	0.384 ^{a} ± 0.02

Data are represented as means ± S.E. Means
The same letter in the same row is not significantly different at $P < 0.05$

Biochemical parameters:

The plasma total protein concentration were increased significantly in all group of fish fed diet contained the *Spirulina* in comparison with control group (Table 6). These results illustrate that the anabolic effect of *Spirulina* on the improvement of the nutritional value of the diet. Where the level of globulin in plasma of all supplemented fish group was increased significantly than those of the control group. While, the Albumin concentration in all fish groups exhibited none significant variation than those of the control group. On the hand, the ratio between Albumin and

globulin (A/G) was decreased significantly in fish fed with all level of *Spirulina* (Table 6).

Table 6: The changes of Total protein, Albumin, Globulin and ratio A/G in plasma *O. niloticus* fed in diet contain *Spirulina platensis* additives and with or without injection of Pathogenic bacteria *Pseudomonas fluorescence.*

Items	Control (0.0 ‰ Spirulina)	Treatment 1 (5‰ spirulina)	Treatment 2 (10 ‰ Spirulina)	Treatment 3 (15‰Spirulina)
Protein (g/100 ml) in plasma of *O.niloticus*				
Non infected	$1.91^{c} \pm 0.034$	$2.834^{ab} \pm 0.38$	$2.27^{b} \pm 0.302$	$3.13^{a} \pm 0.37$
Infected	$1.32^{c} \pm 0.17$	$2.44^{a} \pm 0.0123$	$2.3^{b} \pm 0.057$	$2.12^{b} \pm 0.267$
Albumin(g/100 ml) in plasma of *O.niloticus*				
Non infected	$0.66^{b} \pm 0.12$	$0.74^{a} \pm 0.17$	$0.68^{ab} \pm 0.11$	$0.71^{ab} \pm 0.13$
Infected	$0.83^{a} \pm 0.21$	$0.76^{ab} \pm 0.26$	$0.73^{b} \pm 0.15$	$0.71^{b} \pm 0.11$
Globulin (g/100 ml) in plasma of *O.niloticus*				
Non infected	$1.125^{c} \pm 0.03$	$2.11^{b} \pm 0.02$	$2.04^{b} \pm 0.03$	$2.42^{a} \pm 0.032$
Infected	$0.49^{c} \pm 0.002$	$1.68^{a} \pm 0.03$	$1.57^{b} \pm 0.04$	$1.41^{b} \pm 0.027$
Albumin / Globulin ratio				
Non infected	$0528^{a} \pm 0.001$	$0.35^{b} \pm 0.02$	$0.333^{b} \pm 0.001$	$0.293^{b} \pm 0.002$
Infected	$1.69^{a} \pm 0.023$	$0.452^{b} \pm 0.01$	$0.465^{b} \pm 0.02$	$0.503^{b} \pm 0.02$

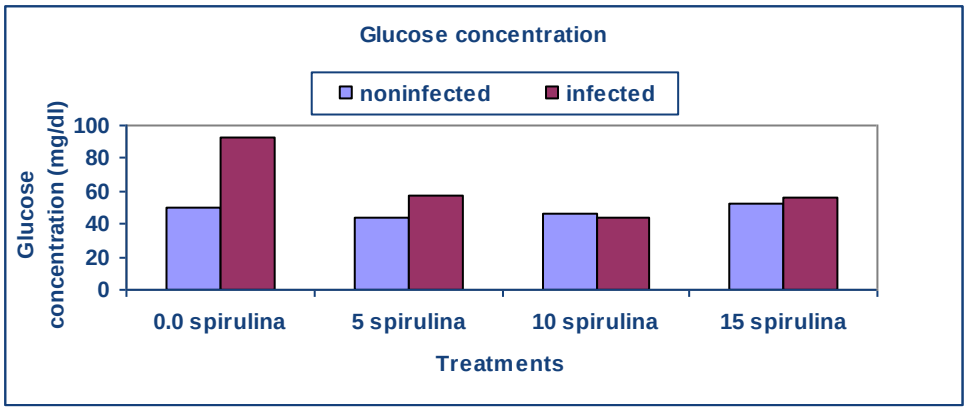
The same letter in the same row is not significantly different at $P < 0.05$.

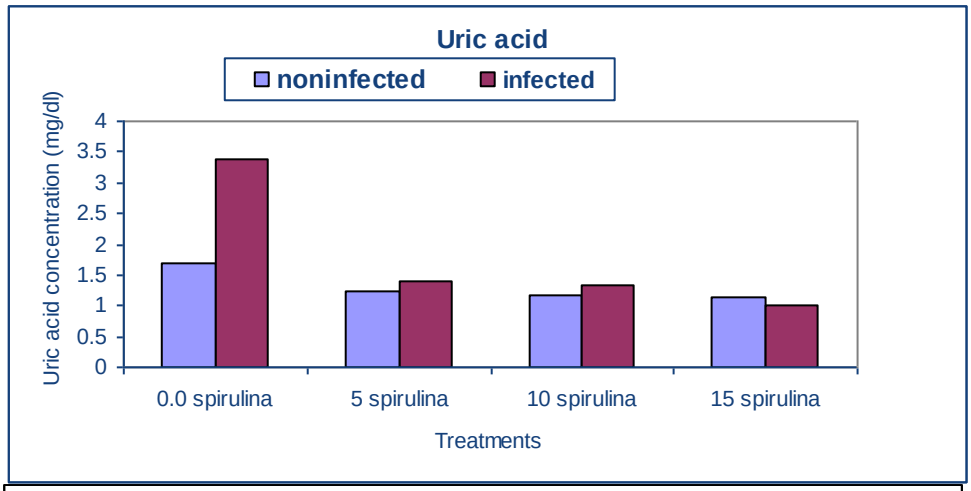
Results in figure (1) showed variations in glucose, uric acid, creatinine in plasma. Data indicated no significant difference in plasma glucose in non infected fish. While, this level was decreased significantly in the blood plasma in *O.niloticus* that injected with *Pseudomonas fluorescence* when compared with control group with (IP) infection of *Pseudomonas fluorescence* (92.2mg/100ml). Similarly The highest value of plasma uric acid was observed in the non infected and infected fish

control (2.69 and 3.38 g/100ml, respectively), While it decreased significantly in all fish groups fed on diets containing 5,10, 15 g *Spirulina* / kg diet. . On the other hand, the creatinine level was significantly decreased with increasing *Spirulina* levels in the tested diets.

The changes in alinine aminotransferase (ALT) activity in plasma are shown in figure (2), ALT activity decreased significantly with increasing levels of Spirulina diet. The highest values were obtained in control group (non infected and infected fish) to 24.8 and 38.2 IU/L respectively. Similarly, Plasma asparate aminotransferase (AST) activity in *O niloticus* after fed with Spirulina and Spirulina with (IP) infection of *Pseudomonas fluorescence* was varied significantly between control groups, whereas a significant reduction (12.6±1.56, 13.4±3.26, 11.4±1.63 IU/l) was recorded in fish fed on diets containing 5, 10and 15 g Spirulina, respectively, $p<0.05$. The same happened with fish injected by Pseudomonas fluorescence with different level of Spirulina.

Fig (1): The changes of glucose (mg/L), uric acid (g/100ml) and creatinine in plasma of *O. niloticus* fed in diet contain *Spirulina platensis* additives and with or without injected Pathogenic bacteria *Pseudomonas fluorescence.*





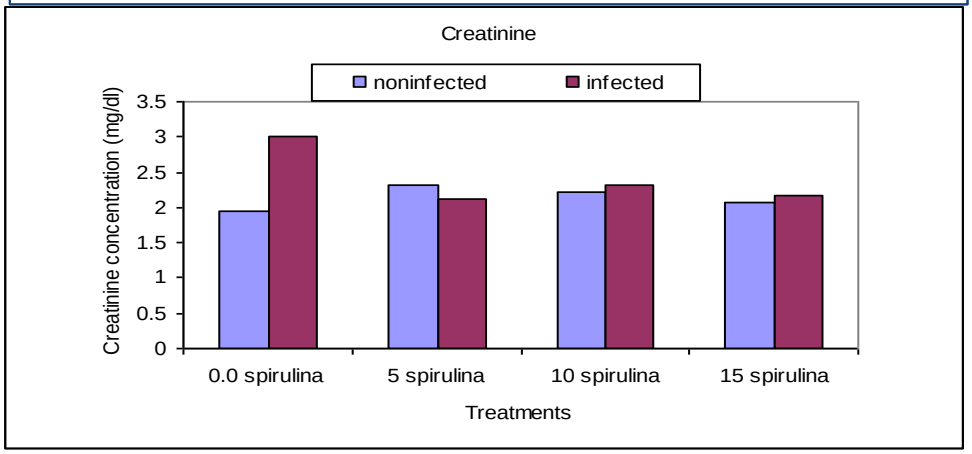
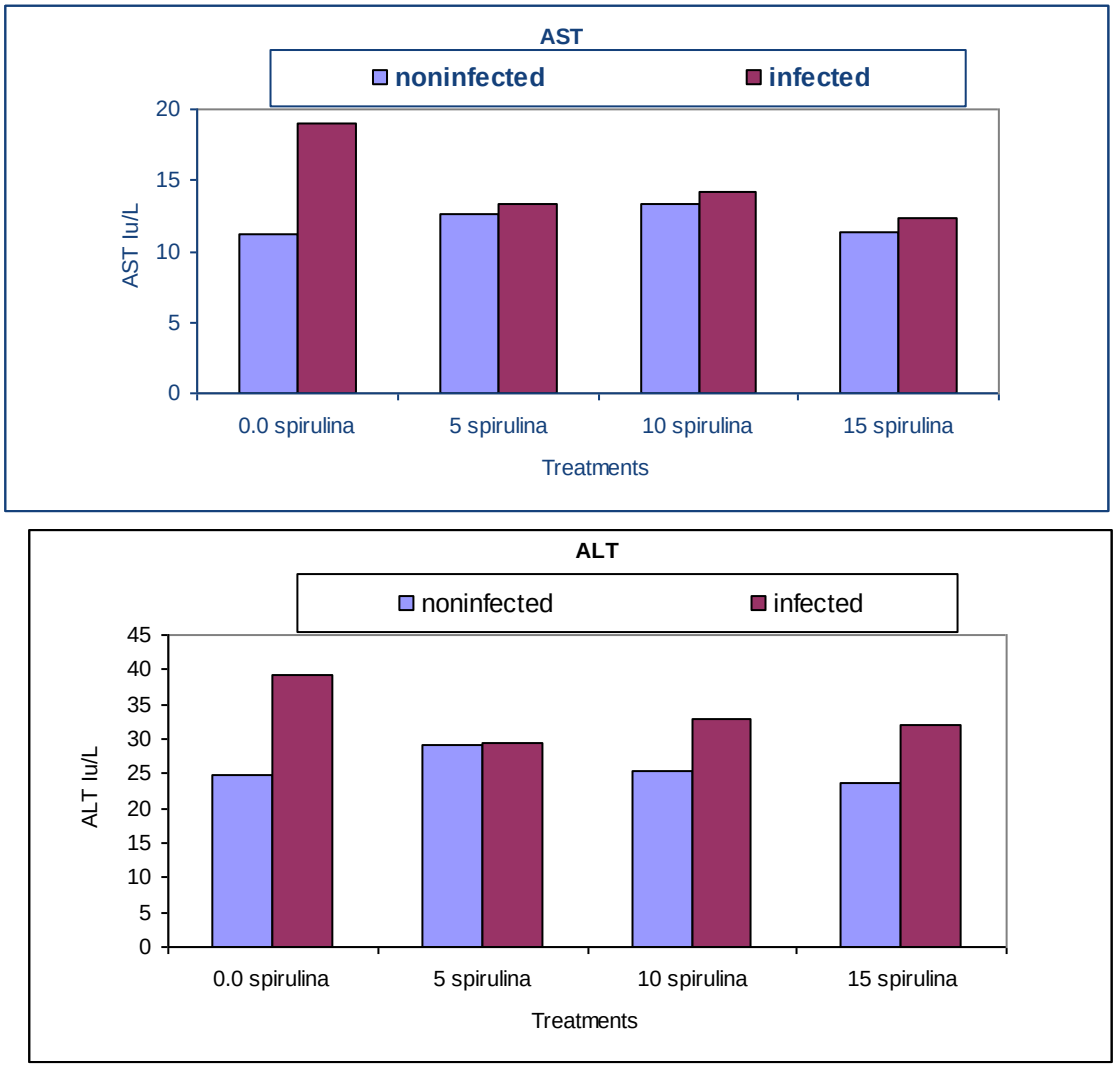


Figure (2): Changes in alanine amino transferase and aspartate amino transferase (Iu/L) in plasma of Nile tilapia (*O.niloticus*) fed diet containing different levels of *Spirulina platensis* and with or without infected by Pathogenic bacteria *Pseudomonas fluorescence*.



Challenge test: fish mortality after IP injection with *Pseudomonas fluorescence* increased significantly ($p<0.05$) by increasing the rearing time and the maximum fish mortality rate was obtained 4 days post

bacterium IP injection after which no mortality was observed (Table 7). The total fish mortality for 14 days after (IP) infection with *Pseudomonas fluorescence* decreased significantly ($P<0.05$) with the increase of *Spirulin*a supplemention. Concerning the challenge of the *O. niloticus* fish groups (control and groups supplemented with Spirulina groups) with specific fish pathogenic *Pseudomonas fluorescence*. The results indicated that appearance of characteristics clinical signs and post mortem lesions in *O. niloticus*, control groups as early as three day post challenge as a total mortality percentage 30%. The clinical signs to the effect of extracellular proteases produced by *Pseudomonas fluorescence* which attack endothelial lining of blood vessels and parechymatouus organ causing the haemorrhage phenomena as well as the degenerated changes in the tail and fin rot.

On the other hand, the *O. niloticus* in groups supplemented by *Spirulina* showed high survival rate within the experimental period post challenge than control groups

Table 7: Mortality fish *O. niloticus* fed in diet contain *Spirulina platensis* infected with Pathogenic *P.fluorscence*

Items	Control (0.0 ‰ Spirulina)	Treatment 1 (5‰ spirulina)	Treatment 2 (10 ‰ Spirulina)	Treatment 3 (15‰Spirulina)
Total No. of Fish	50	50	50	50
No. of fish dead	15	3	1	0
Survival rate (%)	70	94	98	100
Mortality rate (%)	30	6	2	0.0

DISCUSSION

Spirulina provides phycocyanin, a source of biliverdin which is among the most potent of all intra-cellular antioxidants. *Spirulina* is a powerful tonic for the immune system. In studies on mice, hamsters, chickens, turkeys, cats and fish, *Spirulina* consistently improves immune system function. *Spirulina* contain phycocyanin (14%) chlorophyll (1%) and caroteoids (orange/ red 47%) pigments 30. B-carotene of *Spirulina* maintains the mucous memberane firmly and thereby entry of toxic element into the body is prevented. Chlorophyll of *Spirulina* acts as cleansing and detoxifying phytonutrient agansit toxic substance. Now there is strong evidence that *Spirulina* supplements the amount of unconjugated biliverdin which the fish or other animals are born with, providing profound protection from oxidative stress (Strohmeyer, 2009).

The current experiment aimed to study the effects of supplementation of *Spirulina* in the diet of Nile tilapia. *Spirulina* was used at the levels 5, 10, 15 g/kg diet and the data given in table (2-3). Indicate that the final weight, weight gain, and SGR increased significantly with all treatments of *Spirulina.* The highest growth performance was observed in fish fed on 15g *Spirulina.* These results partially agree with those mentioned by Richmond (1988), Ezzat *et al.,* (2005), El Gammal (2005) and Abdel-Tawwab *et al.,* (2008). They found that feeding *Spirulina* to fish improved survival and growth rates and group highest value of PCR and lowest value of FCR. This may be due to the cellular walls of *Spirulina platensis* are mad of digestible muco-proteins (Richmond 1988) having a digestibility value of 83-84% Stantillan, 1979). The capacity to digest microalgae protein is explained by the low pH in herbivorous fish stomachs, which allows them to leach nutrients from cell without breaking its wall (Horn and Messer, 1992). This process would be very easy for tilapia considering that its stomach has the lowest pH level among the fish (Ekpo and Bender 1989). Also,

James *et al*., (2009) showed that supplementation of *Spirulina* in the diet improved the food utilization parameter in carp, *Cirrhinus mrigala* after exposed to copper.

Carcass quality is a matter of great importance from the perspective of consumer acceptance. It has been reported that carcass composition for fed fish diets supplemented with *Spirulina platensis* was of higher quality with less fat and high protein content than control, the same trend also observed in ash content. These results are in agreement with those obtained by Liao *et a*l., (1990), Watanabe *et al*., (1990)' El Gammal (2005), Lin, *et al*., (2007) and Bemejo *et al*., (2008). Nandeesha *et al.,* (2001) reported that the dietary of garlic *spirulina* increased feed intake, FCR, PER, and body composition (crude protein, ether extract, ash, and moisture) in Indian major carps, (*Catla catla*) and rohu (*Labeo rohita*), Shalaby *et al.* (2006) found that the dietary of garlic, *Allium sativum* increased feed intake, FCR, PER, and body composition (crude protein, ether extract, ash, and moisture) in fish.

The present study revealed that administration of *Spirulina* induced significant increases in all blood parameters (erythrocyte count, haemoglobin content and haematocrit value) in treated fish, which agrees with the results of James *et al*., (2009) they verified that the addition of *Spirulina* to fish diets increased erythrocytes number, haemoglobin content, haematocrit value when the carp exposed to 0.63 ppm copper toxicity. The data emphasize the considerable haeamatological changes which take place in *O. niloticus* following challenge with *P. fluorescens*. The pattern of changes was in general, similar to that given following infection of common carp with *P. fluorescens* (Yildiz, 1998). The haematological parameters were improved by challenge with *P. fluorescens* in *O. niloticus* fed *Spirulina* supplemented diet as against infected fish fed spirulina–free diet. It suggests the protective role of *Spirulina* against infection by *P. fluorescens*, in *O. niloticus*. *Spirulnia*

has 14% phycocyanin and it stimulates the erythropotin (EPO) hormone production for haemstopoesis.

Leucocyte is currently used as an indictor of infection disease because it reflects changes in the circulating leucocyte count during infection. In normal fish population, the leucocyte value is low (Boon *et al*., 1990) where as infections activate the proliferation of cells involved in both cellular and humoral immune response (Anderson, 1974). In the present study the total leucocyte count, heterophils, lymphocyte and monocyte number were higher in infected fish fed diet without *Spirulina*. The biological significant of elevated leucocyte is correlated to organism defense against pathogen (Balfry *et al*., 1994).

In the present study, fish fed on diets contain *Spirulina* exhibited higher protein, and decreasing the A/G ratio, as compared with that fed the control diet. This could be attributed to the immuno-modulatory effect of *Spirulina platensis* on the liver cells which activate the anabolic capacity of the hepatocystes to produce blood proteins particularly globulin and increase the glucose concentration as a secondary response to stress by infected *P. fluorescens* (Pottinger and Carrick, 1999). Similar results were obtained by Schaperclaus *et a*l. (1992) and Abdel-Tawwab *et al.* (2008), they started the improvement of fish health when fed Spirulina-supplemented diets. On other hand, the plasma total protein and globulin concentration were decreased significantly in infected fish fed without *Spirulina.* This finding is in agreement with previous work carried out in other fish species (Rehulka, 1996). Moreover, the measurement of albumin, globulin and total protein in plasma is considerable diagnostic value in fish, as it is affected by the general nutritional status as well as the integrity of the vascular system and liver function (Schaperclaus *et al.,* 1992). *Spirulina* spp. in diets acts as an antioxidant (Miranda *et al.,* 1998) immune enhancing (Quareshi *et al*.,

1996) liver protecting (Torres- Duran *et al.*, 1998) and blood lipid lowering effects (Iwata, 1990).

In the present study, plasma glucose concentration was reduced significantly in fish fed on diets containing the highest levels of *Spirulina platensis* (15g/kg diet). This may be due to attributed to *Spirulina* dose which play an important role in the innate immunity of the Nile tilapia, These results agree with those of Ruan *et al*. (2009), they found that white shrimp (*Litopenaeus vannamei*) injected with 0.1, 0.5, and 1 ng./g. (-1) ferritin showed significant decrease of plasma glucose levels, Ferritin play an important roles in the innate immunity of the white shrimp. The same author found that lower levels of plasma glucose in fish have also been reported in the assessment of physiological effects of *Spirulina platensis*.

The uric acid and creatinine level are indicators for kidney function and considered as important variables predicting to which limit the kidney is adversely affected. In the present study, uric acid and creatinine level showed a significant decrease in infected fish fed with *Spirulina*. Infection of Nile Tilapia by *Pseudomonas fluorescence* caused a clear significant increases in the values of plasma uric acid and creatinine level in fish fed on control or treated diets compared with the respective value of non infected fish. In this study, *Spirulina platensis* could stimulate the immune system via increasing the phagocytic and the natural killer activities (Qureshi and Ali 1996). Uric acid and creatinene were decreased after fish fed with *Spirulina*. This may be due to the ability of *Spirulina platensis* to improve kidney functions. This finding agrees with that of Duncan and Klesius (1996) who reported that *Spirulina platensis* are capable of enhancing non-specific immune responses in fish. However, they demonstrated that peritoneal phagocytes from channel cat fish fed *Spirulina platensis*, showed enhanced phagocytosis to zymosan and increased chemotaxis Edwardsiella ictaluri

exoantigen. Watanuki *et al.,* (2006) reported that *Spirulina platensis* activated the functions of leucocytes such as phagocytosis and production of superoxied and cytokines production in common carp.

Transaminases alanine transferase (ALT) and aspartate transferase (AST) are frequently used to diagnose the sublethal damage to different organs. The results of hepatic enzymes analysis (ALT and AST) which decreased in *O. niloticus* plasma kept on supplemented *Spirulina platensis* in comparison to control groups. These results proved the improvement of fish health when fed *Spirulina* supplemented diets and help fish to resist *P. fluorescens* infection. The rise in plasma levels of ALT and AST in Nile tilapia after injected by *P. fluorescens*, may be attributed to the damage structural integrity of the liver, these are normally located in cytoplasm and released into circulation after cellular damage, (Sallie *et al*., 1991). *Spirulina platensis* seems to preserve the structural integrity of hepatocellular reduction *P. fluorescens* induced rise in plasma enzyme in Nile tilapia. The decrease of plasma enzyme in *P. fluorescens* induced liver damage by *Spirulina platensis* may be due the prevention of leakage of intrecellular enzyme by its membrane stabilizing activity. These results partially agree with those mentioned by Sabina *et al.* (2005).

In the present study, the results of bacteria challenge, bacteriocidal activity, and NBT suggest the increase in phagocytosis in blood, which have an important role for prevention of infectious disease. Phagocytosis by these cells is a process of internalization, killing and digestion of invading microorganisms. In phagocytosis, phagocytes produce oxygen free radicals during the respiratory burst, which is toxic to bacteria. Several authors reported that phagocytosis is stimulated by oral administration of probiotics (Choudhury, *et al.,* 2005, Panigrahi *et al*., 2005 and Watanuki *et al.,* 2006).

In the present study, *Spirulina* could stimulate the immune system via increasing the phagocytic and the natural killer activities (Qureshi and Ali, 1996). This finding agrees with Duncan and Klesius (1996) who reported that Spirulina are capable of enhancing non-specific immune responses in fish. However, they demonstrated that peritoneal phagocytes from channel catfish, *I. punctatus* fed *S. plantesis*, showed enhanced phagocytosis to zymosan and increased chemotaxis to *Edwardsiella ictaluri* exoantigen. Watanuki *et al.* (2006) reported also that *Spirulina* activated the functions of leucocytes, such as phagocytosis and production of superoxide, and cytokines production in common carp, *Cyprinus carpio*. The obtained results showed that tilapia fed 5.0 - 15.0 g Spirulina/kg diet increased the fish resistance against *P. fluorescens*. In this regard, Abdel-Tawwab *et al.* (2008) and Watanuki *et al*. (2006) estimated the fluctuation in the number of bacterial cells in Spirulina-treated fish organs after an artificial challenge with *A. hydrophila*. They found that the bacteria numbers were lower in the liver and kidney of carp treated with Spirulina than the control group suggesting the increased resistance of A. *hydrophila* infection.

In conclusion, the results of the present study revealed that the addition of *Spirulina* in the fish diet can promote the growth performance, feed efficiency of Nile tilapia as well as its resistance to *Pseudomonas fluorescence* infection and improve the fish health

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استخدام الأسبريولينا كإضافات غذائية لتحفيز معدلات النمو و تحسين المناعة ضد العدوى البكتيرية للسودوموناس فى اسماك البلطى النيلى

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المعمل المركزى لبحوث الثروة السمكية- العباسة- أبوحماد- شرقية.

تمت دراسة تأثير الأسبريولينا كمنشط للنمو وتأثيره على بعض التغيرات الفسيولوجية وآداء النمو ومعدل البقاء فى إصبعيات أسماك البلطى النيلى وزن (٣.٣±٠.٢ جم). وتم توزيع التجربة إلى ٤ معاملات وتركت المجموعة الأولى كمجموعة ضابطة حيث تم تغذيتها بعليقة متزنة تحتوى على ٣٠% بروتين خالية من الأسبريولينا بينما المجموعة الثانية والثالثة والرابعة تم تغذيتها بنفس العليقة مع اضافة الأسبريولينا بمستويات مختلفة ٥، ١٠، ١٥ جم/ كجم للعليقة للمعاملات الثلاثة على التوالى. وبصوره عامه وتم تغذية الاسماك لمدة ٦ أيام أسبوعيا خلال فترة التجربة ٩٠ يوما. وبعد اجراء القياسات الخاصة لمعدلات النمو والتحليلات اللازمة . تم حقن ٥٠ سمكة لكل معاملة ببكتريا السودوموناس وتركت الأسماك لمدة ١٤يوما أخرى لملاحظة التغيرات المرضية التشخصية ونسبة النفوق ومعدل الأعاشة وثم أجريت التحليلات الازمة مرة اخرى لجميع المعاملات.

وأسفرت النتائج عن الأتى:

١- زيادة الوزن النهائى ومعدل النمو النوعى زيادة ملحوظة بزيادة مستوى الأسبريولينا فى العليقة ووجد أن أعلى معدل نمو فى الاسماك التى تغذت بعلائق تحتوى ٥، ١٠، ١٥ جم من الأسبريولينا /كجم من العليقه لم يحدث وفيات فى كل المعاملات وكما حدث نقص معنوى فى دلائل المناسل و الكبد فى جميع المعاملات الى تم تغذيتها الأسبريولينا وبينما معامل الحالة (Condition factors) لم يظهر اختلافات معنوية فى جميع المعاملات . وجد ان أقل معدلات التحول الغذائىسجلت فى المجموعة المغذاة على الأسبريولينا ١٥جم/كجم عليقة وكذلك كفاءة تحويل البروتين تزداد مع زيادة الأسبريولينا بالعليقة. كما حدث زيادة معنوية بالمحتوى البروتينى والرماد فى جسم الاسماك بينما انخفض محتوى الدهن للاسماك المغذاة على علائق محتوية ٥، ١٠، ١٥ جم الأسبريولينا/ كجم عليقة.

٢- لوحظ اختلاف فى بعض معايير الدم بشكل ملحوظ حيث أظهرت النتائج تأثيرا ذا دلالة معنوية على كرات الدم الحمراء والبيضاء و والخلايا الليمفوية والخلايا الملتهمة والهيموجلوبين، الهيماتوكريت (Hct) فى الأسماك المعداه والغير معداه.

٣- كما حدث زيادة معنوية فى المحتوى البروتينى والجلوبيولين لبلازما الدم فى جميع المعاملات المغذاة بالأسبريولينا بالأسماك المعدية والغير معدية بينما حدث عكس ذلك فى الليبومين وحمض يوريك والكريتيين و جلوكوز الدم بنفس المعاملات. وكذلك تباين نشاط أنزيمات AST, ALT فى البلازما للمعاملات المختلفة للاسماك المعدية والغير معدية عند مقارنتها بالمجموعة الضابطة لكل حالة.

٤- كانت نتيجة اختبار التحدى بحقن بكتيريا االسيدوموناس ٠.٣x ١٠ ^{٧} خلية لكل ملى (بالحقن البريتونى) ووجد عدم حدوث نفوق فى الأسماك مغذاة ١٥ جم بالأسبريولينا/ كجم وزيادة نسبة الأعاشة الى ١٠٠ % فى نفس المجموعة المغذاة بعليقه تحتوى على ١٥جم الأسبريولينا/ كجم عليقة.

٥- وبذلك يوصى باضفة طحلب الأسبريولينا على علائق الأسماك لأنها قد تحسن من إنتاجية الأسماك ومعدل الأعاشة وتحسين مقاومتها للأمراض.