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EVALUATION OF DIETARY SUPPLEMENTATION OF SPIRULINA (ARTHROSPIRA PLATENSIS) ON GROWTH AND SERUM BIOCHEMICAL PARAMETERS OF NILE TILAPIA (OREOCHROMIS NILOTICUS)

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ABSTRACT

A 90-day feeding trial was undertaken to evaluate the effect of adding Spirulina, (Arthrospira platensis) (AP) to the diet of 90 (Oreochromis niloticus) fingerlings weight (21.44±0.31 gm) on growth parameters and health status. Two iso-nitrogenous (30 % crude protein); iso-energetic (3000 Kcal) formulated diets divided into: basal diet without any addition (D1), and basal diet with 0.6 gm Spirulina /kg diet (D2). Each treatment was randomly stocked into 6 glass aquaria (75 L each) with three replicates (n=15) where growth parameters measured every month while serum biochemical components were measured at the end of feeding trails. The results showed that in comparison with D1, Fish body weight gain was not significantly (P≥0.05) affected by addition of 6 g/ Kg of AP/ Kg diet (D2). Feed intake of Spirulina supplemented group was significantly (P<0.05) decreased. Consequently, FCR was not favorable in supplemented group. Protein intake and protein efficiency ratio decreased significantly during second month as compared with D1. However, protein intake increased significantly at the third month compared to D1. Also SGR%/day in D2 was not significantly (P≥0.05) affected. Also there had been no significant (P=0.451) differences in dry matter and ash between D2 or D1, but D2 was the better protein content than D1.

Serum biochemical components as lysozyme, (μg/ml), total protein and globulin of Oreochromis niloticus were significantly (P<0.05) increased in D2 supplemented group after three months of experimental period compared with the D1 supplemented group.

Keywords: Arthrospira platensis, growth performance, Oreochromis niloticus, Lactobacillus.

INTRODUCTION

The aquaculture industry has been globally recognized as the fastest growing food producing industry (FAO, 2014). Fish are an important economic source of protein compared to other sources of animal protein. In developing countries, fish contribute about 30% of the total consumption of animal protein per capita (Wang *et al*., 2017). Aquaculture contributes more than half of the total fish production in the world (Subasinghe *et al*., 2009). In Egypt, the aquaculture industry provides about 77% of the total national fish production and secures more than 580,000 jobs for workers in this sector (GAFRD, 2014; Mur, 2014; El-Sayed *et al*., 2015; FAO, 2016a, FAO, 2016b).

The major threatening problems faced by Nile tilapia are diseases that cause severe financial loss. Although Nile tilapia (*O. niloticus*) is somewhat more resistant to diseases than other kinds of fish, many pathogenic organisms still affect them, threatening their production (Farmer and Hickman-Brenner 1992). So to control those problems we must research for alternative dietary components, such as functional food, in order to reduce the need for antibiotics in aquaculture (Perez-Sanchez *et al.*, 2011).Functional foods may be designed to have physiological benefits and /or reduce the risk of chronic disease elsewhere basic nutritional functions, and may be similar in appearance to conventional food and consumed as part of a regular diet (Gouveia *et al.*, 2010). Examples of functional foods are phytochemicals or other antioxidants and probiotics.

Spirulina (*A.platensis*) is a source of important biological components such as allophycocyanin and β-Carotene, phycocyanin antioxidant activity, sulphated polysaccharides giving antiviral properties, and sterols, which are responsible for anti-microbial activity (Wang *et al.*, 2007). *Spirulina* yields an immunostimulating effect by increasing the resistance of humans, and fish to infectious diseases, the capacity of influencing hemopoiesis, stimulating the production of antibodies and cytokines. Also *Spirulina* is a source of high biological value protein (60–70% crude protein) (Lordan *et al.*, 2011), minerals (especially iron), essential fatty acids and vitamins (especially vitamin B12 and pro-vitamin A), (Gouveia *et al.*, 2010).

Spirulina has been used at a low-level as a feed additive to improve the taste, texture or color of the whole fish or its flesh and for its positive effects on growth, physiological condition, feed utilization and stress and diseases resistance (Mustafa and Nakagawa 1995). Therefore, the main objectives of this study were:

1. To investigate the effects of dietary supplementation of *Spirulina*, (*A. platensis*) on growth performance, feed utilization.
2. To evaluate immunity competence as *Spirulina*, (*A. platensis*) has antimicrobial activity.
3. And to evaluate the effect of dietary supplementation of *Spirulina* (*A. platensis*) on microbial load inside Nile tilapia (*O.niloticus*) fingerlings.

MATERIALS AND METHODS

Experimental Work

The present study was conducted at the Department of Internal Medicine, Infectious Diseases and Fish diseases and Management, Faculty of Veterinary Medicine, Mansoura University, Egypt. This study aimed to investigate the effects of dietary supplementation of *Spirulina* (*A. platensis*) on growth performance, health status and intestinal microflora of Nile tilapia (*O. niloticus*) fingerlings reared in aquarium for 90 days.

Experimental System and Fish

Six glass aquaria with dimensions of 100×30×40 cm were used. The experimental treatment was tested in three aquaria for each where each aquarium contains 15 fish. Each aquarium was marked by 3 marks (top, middle and bottom marks) and filled with dechlorinated tap water to the top mark (25 cm) and this gives a water volume of 75 liters. Every day 8 liters of water was removed (middle mark) and 10 liters of dechlorinated fresh tap water was added. At the day of fish weighing, after removal of the fish from the aquarium by net, 20 liters of the water of the aquarium was removed and equal amount of dechlorinated fresh water was added. Each aquarium was supplied with automatic heater (to maintain the water temperature at 28 ± 1˚C), air pump and stone (to provide continuous aeration to water). The dissolved oxygen was 4-6 ppm. Water pH was in the range of 7.2 -7.5 during the experimental period .Nile tilapia (*O. Niloticus*) fingerlings was obtained from Governmental Fish Hatchery, El Manzala, Dakahlia, Egypt and transported to the wet lab at the Department of Internal Medicine, Infectious Diseases and Fish diseases and Management, Faculty of Veterinary Medicine, Mansoura University, Egypt. Fish were fed control diet containing 30% crude protein and 3000 Kcal for 14 days as a conditioning period before stating the experiment.

Sources of *Spirulina*

Spirulina (*A. platensis*) was collected from Al Hamra Lake, Wadi El-Natron, El Beheira Governorate, and was developed in 20 liter plastic bottles in zarouq environment, dried and grinded in Fish Production Lab. Animal and Fish production Department, Faculty of Agriculture, Alexandria University.

Experimental Feeding Trails on Nile Tilapia Fingerlinges

An experimental feeding trial was conducted using Nile tilapia (*O. niloticus*) fingerlings to evaluate the effects of supplementing (0.0, and 6 g /kg diet) of *Spirulina* (*A. platensis*) on growth performance, feed utilization and whole body composition, health status of Nile tilapia (*O. niloticus*) fingerlings.

Experimental Diets

For formation of the experimental diets (30% crude protein and 300 kcal) the following ingredients were used: fish meal, Corn gluten, soybean meal, wheat bran, Rice bran, yellow corn, corn oil, vitamin and mineral premix. The amount of diet required was calculated according to the weight of fish used, growth expected dietary regime and experimental period. Fine ingredients were weighed out together and thoroughly mixed, followed by the addition of corn oil and further mixing for 10 min. Distilled water (300 ml/kg) was then added to soften the mixture and this was passed through mincer with a 3 mm (diameter) and the pellets were dried in an oven at 65 ˚C. The dried diets were broken up into convenient pellet size. Fish in each aquarium were fed experimental diets twice a day, six days a week at a rate of 3% of their body weight.

Proximate Chemical Analysis:

Samples of the experimental diets and fish were chemically analyzed to determine dry matter (DM), crude protein (CP), and ether extract (EE), crude fiber (CF), and ash contents according to the methods of AOAC (2000). Nitrogen free extract (NFE) was calculated by differences. Gross energy (GE) contents of the experimental diets and fish samples were calculated by using factors of 5.64, 9.44 and 4.12 kcal/g of protein, lipid and carbohydrates, respectively (NRC, 1993). Proximate analysis composition of the experimental diets was presented in Table (1).

Fish Performance, Feed Efficiency

Fish growth performance and feed efficiency parameters were calculated according to Cho and Kaushik, (1985) as following equations:

1) Body weight gain, (BWG g / fish) = [final body weight-initial body weight]

2) Body weight gain, (BWG, %) = [(W2-W1/W1) X100] where W2 and W1 is the final and initial weight respectively.
3) Specific growth rate (SGR% / day) = [Ln final weight - Ln initial weight] × 100 /time (days)
4) Feed conversion ratio (FCR) = feed intake (g)/body weight gain (g);
5) Protein efficiency ratio (PER) = gain in weight (g)/protein intake in feed (g).

Biochemical Parameters

At the end of the experimental period (90 days feeding trials), fish were not fed for 24 h prior to blood sampling. Five fish from each aquarium were taken for physiological investigation. Fish were anaesthetized using buffered tricaine methanesulfonate (20 mg/L), and blood was collected from the caudal vein with a sterile syringe and divided equally among two clean and dry tubes. The first part was centrifuged at 3,000 rpm for 15 min and the serum was stored at −20 ◦C for total protein, albumin and globulin content determination colorimetrically using spectrophotometer kit (purchased from Bio-diagnostic, Egypt) according to the manufacturers' instructions (Folin and Wu, 1920; Fawcette and Scott, 1960;Henry, 1968; Annino, 1976).

To study immunity stimulating capacity, a lysozyme activity assay was conducted (Jongkon Promya and Chanagun Chitmanat, 2011). Shortly, the dry *Micrococcus* sample (0.2 mg mL-1) in a 0.04 M phosphate buffer saline solution (pH 5.75) was used as the substrate where 40 µL of serum from the fish sample was added to 3 mL of the bacteria. The opacity levels at 540 nm decreased after placing the sample at room temperature for 0.5 and 5 min were measured. A unit of lysozyme activity is referred to as the opacity level has decreased 0.001 min^{-1}.

Statistical Analysis

All data were analyzed by one-way ANOVA using the statistical analysis system procedure (SAS, 2006). All percentages and ratios were transformed to arcsine values prior to analysis (Zar, 1984). Duncan's multiple range tests were used as a post hoc test to compare differences among individual means at ($P \leq 0.05$).

RESULTS

Growth Performance And Feed Efficiency

The effect of the dietary *Spirulina platensis* supplementation on growth performance of Nile Tilapia (*Oreochromis niloticus*) is summarized in (Table 2). At the end of experimental period there was no significant difference in the body weight gain between experimental diets. Also in all experimental periods, body weight gain% was not significantly ($P \geq 0.05$) increased in fishes of D2 supplemented diet compared with D1. Average specific growth rate (expressed as SGR%/day1) was not significant increased ($P \geq 0.05$) with D2 supplementation in all experimental periods.

Also feed efficiency of experimental fish is summarized in (Table 3). Data show that D2 was significantly lower feed intake during second month than D1. Also FCR of D2 was not significantly improved ($p \geq 0.05$) in all experimental periods compared to D1. Also D2 showed no increase in protein intake during all experimental period ($P > 0.05$). Protein efficiency ratio and weight gain % not affected during all experimental periods compared to D2.

Table (1) Ingredients and proximate composition of formulated diets.

Ingredients	Formulated diets	
	D1	D2
Fish meal	9.30	9.30
Gluten	13.00	13.00
Soybean meal	25.00	25.00
Wheat bran	15.00	14.40
Rice bran	15.00	15.00
Yellow corn	18.70	18.70
Sun flower oil	2.00	2.00
Vit. and mineral premix[1]	2.00	2.00
Spirulina platensis meal	0.00	0.60
Total	100	100
Chemical composition (%)		
Dry Matter	93.22	93.32
Crude Protein	30.96	30.7
Crude Fiber	3.48	3.46
Ether Extract	4.31	4.31
Ash	4.66	4.85
NFE[2]	49.81	50
GE kcal/g[3]	4.20	4.19
DE kcal/g[4]	3.15	3.14
P/E (mg/kcal)[5]	9.828	9.777

1 Each 1kg of premix contain: vit A 550000 IU, vit D 110000 IU, vit E 11000 mg, vit K 484 mg, vit C 50 gm, vit B1 440 mg, vit B2 660 mg, vit B3 13200 mg, vit B5 1100 mg, vit B6 1045 mg, vit B9 55 mg, Choline 110000 mg, Biotine 6.6 mg, iron 6.6 gm, copper 330 mg, Mn 1320 mg, Zn 6.6 gm, Se 44 mg, iodine 110 mg .
2 Nitrogen free extract = Organic matter (OM) - (protein + lipid + fiber)
3 Gross energy (GE) was calculated as 5.65, 9.45 and 4.11 kcal/g for protein, lipid and NFE, respectively NRC, (1993).
4 DE (Digestible energy) was calculated by applying the coefficient of 0.75 to convert gross energy to digestible energy according to (Hepher et al., 1983).
5 P/E (protein energy ratio) = crude protein × 1000 / digestible energy, according to (Hepher et al., 1983).

Table (2): Growth performance of Nile tilapia (*O.niloticus*) fingerlings fed on experimental diets containing different levels of *Spirulina* (*A. platensis*).

Items	Experimental diets		*P*-Value
	D1	D2	
Initial body weight (IBW,g/fish)	21	22.83	NS
Final body weight (FBW) ,g/fish	36.99^{b}	38.33^{ab}	NS
Body weight gain (BWG) ,g/fish			
First Month	5.54±0.72	5.00±0.91	NS
Second Month	4.68±0.25	4.95^{a}±1.08	NS
Third Month	5.77^{a}±1.25	5.55±0.81	NS
Body weight gain (BWG), (%)			
First Month	26.38^{a}±3.25	21.90±1.56	NS
Second Month	17.63^{a}±2.37	17.78±3.06	NS
Third Month	18.48^{a}±2.10	16.93±2.50	NS
Specific growth rate (SGR/day/%)			
First Month	1.67±0.04	1.41^{b}±0.06	NS
Second Month	1.16±0.02	1.17±0.05	NS
Third Month	1.21±0.001	1.11±0.02	NS

[1]Diet 1 (control diet without AP); diet 2, contained 6.0 g /kg Spirulina (*A. platensis*),
[2]Means in the same row having different superscripts are significantly different ($P < 0.05$).

Table (3): Feed intake , feed conversion ratio , Protein intake and Protein efficiency ratio of Nile tilapia (*O.niloticus*) fingerlings fed on experimental diets containing different levels of *Spirulina* (*A. platensis*).

Items	Formulated diets		*P*-Value
	D1	D2	
Feed intake (FI,g/fish)			
First Month	19.11±0.46	20.36±0.054	NS
Second Month	24.47[a]±0.60	22.88[b]±0.92	NS
Third Month	18.00±1.85	18.8±0.523	.0001
Feed conversion ratio			
First Month	3.449±0.39	4.073±0.25	0.35
Second Month	5.229±0.06	4.622±2.28	NS
Third Month	3.119[b]±0.51	3.387[a]±0.47	0.05
Protein intake (PI,g/fish)			
First Month	5.916±0.14	6.302±0.15	NS
Second Month	7.508[a]±0.19	7.082[b]±0.28	NS
Third Month	5.574[b]±0.57	5.820[a]±0.16	NS
Protein efficiency ratio (PER)			
First Month	0.936[a]±0.10	0.793[b]±0.12	NS
Second Month	0.623±0.02	0.699±0.14	.0001
Third Month	1.035±0.08	0.953±0.11	NS

[1]Diet 1 (control diet without AP); diet 2, contained 6.0 g /kg Spirulina (*A. platensis*),
[2]Means in the same row having different superscripts are significantly different ($P < 0.05$).

Serum Biochemical Componets

Records of serum biochemical components confirmed that the concentration of serum Lysozyme activity, overall protein, albumin and globulin of D2 were higher (P<0001) than of the manage organization (table 4) (Folin and Wu, 1920; Fawcette and Scott, 1960; Henry, 1968; Annino, 1976). D2 confirmed a better lysozyme (405.67, P< 0.0001), overall protein (3.670, P< 0.0001), albumin (1.665, P< 0.0001) and tended to increase globulin (1.665, P= 0.0001) compared to D1. Consequently, the impact of D2 on serum α1, α2, β1, β2, α1 and α 2 globulin values of fingerlings Nile Tilapia presented in table 4. A great, dose-dependent lower (P > 0001) of serum α1- glob% and γ_{1-} glob% have been found in D2. An opposite effect became noticed regarding α2- glob%, β1- glob%, β2- glob% and γ_{2-} glob,% where the values had been drastically (P<0.0001) extended with addition of *S.platensis*.

Table (4): Serum biochemical values of fingerlings Nile Tilapia fed on experimental diets

Item	Experimental diets (g / Kg feed)		P value
	D1	D2	
At The End Of Experimental Period			
Lysozyme, (µg/ml)	273.00[b]±2.517	405.67[a]±1.764	0.0001
TP, (mg/dl)	2.460b[b]±0.006	3.670[a]±0.012	0.0001
Alb, (mg/dl)	1.085[b]±0.003	1.665[a]±0.005	0.0001
Glob, (mg/dl)	1.42[b]±0.003	1.665[a]±0.007	0.0001
α_{1-} glob,%	34.60[a]±0.002	26.73[b]±0.006	0.0001
α_2- glob,%	5.39[b]±0.001	12.10[a]±0.001	0.0001
β_{1-} glob,%	3.96[b]±0.002	8.03[a]±0.006	0.0001
β_{2-} glob,%	3.89[b]±0.003	4.55[a]±0.001	0.0001
γ_{1-} glob,%	4.60[a]±0.005	2.08[b]±0.003	0.0001
γ_{2-} glob,%	5.22[b]±0.001	7.25[a]±0.001	0.0001

[1]Diet 1 (control diet without AP); diet 2, contained 6.0 g /kg Spirulina (*A. platensis*),
a - d Means in a row not sharing the same superscript differ significantly.

Nile Tilapia Whole Body Chemical Composition

The effect of supplemented *S. platensis* by 6 g SP/kg meal on carcass chemical composition (%) of fingerlings Nile Tilapia is offered in table 5. outcomes confirmed that there had been no significant (P=0.451and 0.358) differences in dry matter and ash among D2 or D1. D2 the better protein content than D1. The lipid content material of Fingerlings Nile Tilapia was notably reduced (p=0.01) with supplementing 6 g SP/kg.

Table (5): Average muscular chemical composition of Nile tilapia after feeding different treatments at the end of the experimental period

Items	Experimental diets (g / Kg feed)		*P*-Value
	D1	D2	
Moisture %	74.10±16.11	74.97±13.11	0.451
Crude protein %	63.10^{b}±23.6	69.90^{a}±3.64	0.005
Crude fat %	20.11^{a}±2.10	18.01^{b}±1.35	0.01
Crude ash %	13.77±0.93	13.68±0.91	0.358

[1]Diet 1 (control diet without AP); diet 2, contained 6.0 g /kg Spirulina (*A. platensis*),
a - c Means in a row not sharing the same superscript differ significantly (p ≤ 0.05).

DISCUSSION

Growth Performance

In our study, there was no significant differences in body weight were observed in experimental diets. But in other hand, a research study on Nile tilapia (Zhao et al., 2006) showed a higher growth in Nile tilapia fed on low levels of algae meal in diets. Where improved growth performance of Nile tilapia fed on diet containing 1% *Spirulina* when compared with the control diet, (Belal et al., 2012) Furthermore, Allam, (2007) showed that 3% algae had a significant (*P<0.05*) effect on body weight of Nile tilapia (*Oreochromis niloticus*) all over the experimental period. It could be recommended that low levels of *Spirulina* (lower than 6 gm/kg) are the most effective for improving the body weight of *Oreochromis niloticus*. But in other studies (Simanjuntak and Wibowo, 2016) in which the finest growth of gurami is obtained at the level of 0.6 g/kg *S. platensis* of diet. These may be due to *Spirulina* when incorporated in the diets, have the beneficial effects on host's gut micro flora. Improvement in growth of fish by dietary inclusion of *Spirulina* has been reported earlier in a number of studies (Tongsiri et al., 2010, Al-Koye, 2013). This finding is in consistent with research Zeinab, (2015); the addition of *Spirulina* in Nile Tilapia diet is very effective in stimulating growth. But in other studies that agree with our results, (Maryam Fakhabi et al., 2015) showed that final average weight, daily growth, body weight of juvenile fishes of sturgeon (*Acipenser stellatus*) increase with the addition *Spirulina* at 0.2 gm algae /Kg diet comparing to other juvenile fishes fed on diets supplemented with 0.3, and 0.4 gm of *Spirulina* algae /kg diet and control group.

The present study suggests that supplementation of fingerlings Nile tilapia diets with up to 6 g SP/kg weight-reduction plan has no effect on feed consumption. This end result is steady with findings of Shimaa Amer, (2016) in Nile tilapia fingerlings, in which inclusion stages 0, 0.5, 1 and 1.5 algae /Kg diet result in no tremendous differences (P>0.05) in feed intake among fish fed *Spirulina* dietary supplements or the control diet.

Consequently, D2 resulted in the lowest value FCR ($p>0.05$). But other studies which in conflict with our results proved that the improved growth rate and FCR after addition of *Spirulina* may be due to the antioxidant and vitamins content of *Spirulina*, which improve viability and FCR (Ali and Saleh, 2012). Maryam Fakhabi et al., (2015) found that feed conversion ratio of juvenile fishes of sturgeon (*Acipenser stellatus*) improved by addition 0.2g *Spirulina* algae/kg diet comparing to other fishes fed on diets 0.3 and 0.4 g SP/Kg diet or control group. This mean also that addition of *Spirulina* with low level (lower than D2) gives positive effect on FCR.

The present study showed that D2 produces no impact on protein intake during all experimental period; also protein efficiency ratio was declined at the same manner. This end result is probably defined by optimizing protein use for growth, a significant quality given that protein is the most high priced feed nutrient, the development in the biological value of the supplemented diets in these treatments with high population and low nutritional protein demonstrated that *Spirulina* supplements performed more effectively in stress conditions (Ringo et al., 1998, Belal et al., 2012, Al-Koye 2013). These results disagree with findings Ebtehal El-Sayed Hussein et al., (2014) in Nile tilapia where the higher value of PER in diets containing *Spirulina* 50% of the gluten meal protein replacement diets. The lowest feed consumption values were recorded D2. The decrease values of FCR, PER% and PI% received from D2. (El-Sheekh et al., 2014) pronounced that the better feed intake in *S. platensis* supplemented diets may have been due to the elevated fish appetite and flavor ensuing in enhancing the growth rate, FCR and PER%. Even as the lower values of FCR and PER% received from the fish group fed with the diet which containing with 50% of dried *S. platensis*.

Specific growth rate (SGR) is the measuring tools reflecting the fish fitness status under natural and experimental conditions. But in the present study, specific growth rate (SGR%/day) was not significantly increase in D2 as compared with D1. But the positive role of dietary algae on SGR (% /day) was reported by some researchers (Britz, 1996 and Pilot et al., 2014). Generally, these results were in conflict with Nasreen, (2014) who reported that addition 5 gm *Spirulina* /kg diet in fish diet tend to increase significantly in weight gain 6.89, Daily growth rate 0.17, Specific growth rate 0.147, Relative growth rate 15.31 and Food conversion ratio 2.14 compared with control or those fed on 1 and 3 gm *Spirulina* /kg diet.

Serum Biochemical Components

Results concerning Lysozyme revealed that increasing dietary *Spirulina* supplementations led to an increasing in serum Lysozyme activity at 90 days of the experimental periods ($p< 0.001$) in a dose dependent manner. This enhancement in serum immunity occurs due to leukocytes secret lysozyme that attacks the bacterial cell wall, stopping their adhesion and colonization. also lipoproteins and high molecular weight polysaccharide of *Spirulina* cell wall act as ligands to immune cell receptors brought about increase the mRNA stages of numerous chemokines and cytokines result in activation of the immune system (Ravi, Maddaly et al., 2010, Watanuki et al., 2006). results were in agreement with Nath et al., (2012), when P. incisa in Guppy fish (Poecilia reticulata) take a diets supplemented with microalgae found out an increase in stress resistance and the survival rate related to the increase of lysozyme level. Besides, Shimaa Amer, (2016) the level of Lysozyme value was found to be higher than those fed on basal diet. El-Sheekh et al., (2014) stated that stimulation of the immune system when supplement diets with *Spirulina* occur due to increasing the phagocyte and the natural killer activities. (Bermejo et al., 2008) who said that the antioxidant capacities of *Spirulina* protein extract may be due to the bili-proteins inside the microalgae, including phycocyanin so that we are able to use *Spirulina* to enhance the animals protection mechanism by way of supplementing their diets with *Spirulina*.

Serum proteins consisted of globulin that is the essential protein constituent of serum that taken into consideration a part of the immune response where it enters in the formation of antibodies. The values discovered for total protein, albumin and globulin are similar to those stated by Ganong, (2005).Total protein can be used to determine the fitness status of fish and maintaining the key position in the immune response (De Pedro et al., 2005). A fish albumin level performs a crucial function within the functioning of colloidal osmotic stress of the blood and the transportation of chemicals exogenous and endogenous metabolites (Baker, 2002).Total protein, albumin and globulin of gurami obtained from D2, (Simanjuntak et al., 2016). *Spirulina* can improve immunity and health of Nile tilapia where increased levels of *Spirulina* in feed diet increase serum total protein and globulin compared to D1, whereas albumin level was not a significant affected with those supplementation, (Zeinab et al., 2015).

CONCLUSION

The present study concludes that *Spirulina* (*A. platensis*) not significantly affect growth performance and feed efficiency of Nile tilapia fingerlings but in other hand help in improving immunity stimulating capacity and health status at end of experimental period in Nile Tilapia.

REFERANCE

Ali, S.K., and Saleh, A.M. (2012): Spirulina – an overview. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(3), 9-15.

Al-Koye HJ. (2013): Effect of Using *Spirulina* spp. Instead of Fishmeal on Growth, Blood Picture and Microbial load of Common carp *Cyprinus carpio*. MSc Thesis College of Agriculture University of Salahaddin-Erbil Iraq; 131.

Allam, H.Y. (2007): Physiological effects of some additives on growth, blood, constitutes and immunity in Nile tilapia (*Oreochromis niloticus*), Thesis Faculty Agric Univ Assiut.

Annino JS. Clinical chemistry principles and procedures, 4thedn. Little Brown and Company, Boston, 1976.

AOAC. (2000) Official methods of analysis of AOAC. International 17th edition; Gaithersburg, MD, USA Association of Analytical Communities.

Baker, M.E. (2002): Albumin, steroid hormones and the origin of vertebrates. *Journal of Endocrinology,* 175, 121-127.

Belal, E.B., Khalafalla, M.M.E., El-Hais, M.A. (2012): Use of *Spirulina* (*Arthrospira fusiformis*) for promoting growth of Nile Tilapia fingerlings. Afr J Mic Res 2012; 6(35): 6423- 6431.

Bermejo, P., Piñero, E. and Villar, A. (2008): Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of *Spirulina platensis*. Food Chemistry, 110: 436–445.

Britz, P. J. (1996): The suitability of selected protein sources for inclusion in formulated diets for the South African abalone, *Haliotis midae*. Aquaculture 140: 63-73.

Cho, C.Y. and Kaushik, S.J. (1985) Effect of Protein Intake on Metabolizable and Net Energy Values of Fish Diets. In: Cowey, C.B., Mackie, A.M. and Bell, J.G., Eds., Nutrition and Feeding in Fish, Academic Press, London, 95-117.

De Pedro, N., Guijarro, A.E., Lopez-Patino, M.A., Marines-Alvares, R., and Delgado, M. (2005): Daily and seasonal variation in haematological and blood biochemical parameters in tench *Tinca tinca*. *Aquaculture Research,* 36, 85-96.

Ebtehal, E.H., Konrad, D., Deyab, M.S., Deyab, E. and Bong, J.L. (2014): Effect of dietary phosphorus supplementation on utilization of algae in the grow-out diet of Nile tilapia Oreochromis niloticus. Aquaculture Research, 45, 1533–1544.

El-Sayed, A. F. M., M. W. Dickson, and G. O. El-Naggar. Value chain analysis of the aquaculture feed sector in Egypt. Aquaculture, 437: 92–101 (2015).

El-Sheekh, M., El-Shourbagy, I., Shalaby, S. and Hosny, S. (2014): Effect of Feeding *Arthrospira platensis* (*Spirulina*) on Growth and Carcass Composition of Hybrid Red Tilapia (*Oreochromis niloticus x Oreochromis mossambicus*). Turkish Journal of Fisheries and Aquatic Sciences 14: 471-478.

Fawcette JK, Scott JE. Practical clinical biochemistry, 4th edn. Arnold Harold Varley, 1960, 119-122.

FAO, Food and Agricultural Organization, (2014). The State of World Fisheries and Aquaculture. Hasan, M.R and De Silva, S.S; FAO Fisheries Technical Paper. No. 223. Rome, FAO. 2014.

FAO. Rice Market Monitor. Vol. XIX(1). http://www.fao.org/fileadmin/templates/est/COMM_MARKETS_MONIORING/Rice/Images/RMM/RMM_APR16.pdf (2016a). Accessed 14 July 2016.

FAO. The state of world fisheries and aquaculture: Contributing to food security and nutrition for all. Food and Agriculture organization of the United Nations. Rome, Italy. ISSN 1020-5489 (2016b).

Farmer, J.J. and Hickman-Brenner, F.W. (1992) The genera Vibrio and Photobacterium. In The Prokaryotes ed. Balows, A., TruÈper, H.G., Dworkin, M., Harder, W. and Schleifer, K.-H. pp. 2952±3011. New York: Springer-Verlag.

Folin O, Wu H. Span Diagnostic Kits, J Bio Chem. 1920;

9:341-367. GAFRD. General authority for fish resources development. In: Fish Statistics Year Book. Cairo, Egypt: Ministry of Agriculture and Land Reclamation (2014).

Ganong, W.F. (2005): Review of Medical Physiology, 22nd edn, LANGE-Science: New York.

Gouveia, L., Patrícia, Moura, and Narcisa, M.B. (2010): Microalgae – source of natural bioactive molecules as functional ingredients. Food Science & Technology Bulletin Functional Foods 2010. DOI: 10.1616/1476-2137.15884

Hepher, B., Liao, I.C., Cheng, S.H. and Hsieh, C.S. (1983): Food utilization by red tilapia — effects of diet composition, feeding level and temperature on utilization efficiencies for maintenance and growth. Aquaculture, 32, 255–275.

Henry RJ. Clincal chemistry, principles and techniques.Harper and Row, New York, 1968, 664-666.

Jongkon Promya and Chanagun Chitmanat (2011): the effects of *Spirulina platensis* and *cladophora* algae on the growth performance, meat quality and immunity stimulating Capacity of the African sharptooth catfish (*clarias gariepinus*). International journal of agriculture & biology. 10–272/DJZ/2011/13–1–77–82.

Lordan, S., Ross, R.P. and Stanton, C. (2011): Marine bioactive as functional food ingredients: potential to reduce the incidence of chronic diseases. Mar. Drugs 9, 1056–1100.

Maryam, A.F., Mehdi, S., Abolghasem, K., Abbas, A. Z. and Mehdi, S. (2015): Effect of *lactococcus lactis* and *Spirulina platensis* on some growth performances of south caspian stellate sturgeon (*acipenser stellatus*) juveniles, Indian Journal of Fundamental and Applied Life Sciences, Vol. 5 (S3), pp. 1775-1780.

Mur, R. Development of the aquaculture value chain in Egypt. Report of the National Innovation Platform Workshop, Cairo, 19-20 February 2014. Cairo: WorldFish. An Industry Assessment of Tilapia Farming in Egypt (2014).

Mustafa, M.G. And Nakagaw, Y.H. (1995): A review: Dietary benefits of algae as an additive in fish feed, *Isr. J. Aqua*, 47: 155-162.

Nasreen M. Abdulrahman (2014): Evaluation of *Spirulina* spp. as food supplement and its effect on growth performance of common carp fingerlings. International Journal of Fisheries and Aquatic Studies; 2(2): 89-92.

National Research Council. 1993. Nutrient Requirements of Warm water Fishes and Shellfishes. Washington, D.C.: National Academy Press.

Perez-Sanchez, T.; Balcazar, J.L.; Garcia, Y.; Halaihel, N.; Vendrell,D.and I.de Blas. (2011) Identification and characterization of lactic acid bacteria isolated from rainbow trout, Oncorhynchus mykiss (Walbaum), with inhibitory activity against Lactococcus garvieae. J Fish Dis.;34(7):499-507.

Pilot, A.I., Sumi, K.R., Sharker, M.R., Mandal, S.C., Mokhlasur Rahman, M.S. and Alam, M.R. (2014): Effect of different live feeds on the growth performance and flesh quality of Nile tilapia (*Oreochromis niloticus*) fingerlings. Int. J. LifeSc. Bt & Pharm. Res. 2014.

Ravi, M., Lata De.S., Azharuddin, S. and Paul, S.F.D. (2010): The beneficial effects of *Spirulina* focusing on its immunomodulatory and antioxidant properties. Nutrition and Dietary Supplements, 2: 73–83.

Ringo, E., Gatesoupe, F.J. (1998): Lactic acid bacteria in fish: a review. Aquaculture ; 160: 177-203.

SAS (2000): Statistical analysis, SAS. STAT user guide version 6.2, SAS instit. INC.,cary, NC.

Shimaa A. Amer. (2016): Effect of *Spirulina platensis* as feed supplement on growth performance, immune response and antioxidant status of mono-sex Nile Tilapia *(Oreochromis niloticus)*. BENHA VETERINARY MEDICAL JOURNAL, VOL. 30, NO. 1:1-10.

Simanjuntak, S.B.I., Wibowo, E.S. and Indarmawan (2016): Stimulation of Deprivation Cycles with *Spirulina platensis* Feed Supplementation on *Osphronemus gouramy* Physiological Responses. Biosaintifika 8 (3) 378-385.

Subasinghe, R., D. Soto, and J. Jia. Global aquaculture and its role in sustainable development. Rev. Aquacult., 1: 2–9 (2009).

Tongsiri, S., Mang-Amphan, K, and Peerapornpisal, Y. (2010): Effect of replacing fishmeal with *Spirulina* on growth, carcass composition and pigment of the mekong giant catfish. Asian J Agricult Sci ; 2(3):106-110.

Wang, L., Pan, B., Sheng, J., Xu, J. and Hu, Q. (2007): Antioxidant activity of *Spirulina platensis* extracts by supercritical carbon dioxide extraction. Food Chemistry, 105: 36-41.

Watanuki, H., Ota, K., Malin, A.C., Tassakka, A.R., Kato, T. and Sakai, M. (2006): Immunostimulant effects of dietary *Spirulina platensis* on carp, *Cyprinus carpio*, Aquaculture. 258: 157–163.

Zar J.H., 1984. Biostatistical Analysis, 2nd ed. Prentice Hall, Englewood Cliffs, NJ. 718 pp.

Zeinab, A.K., Aly, M.S, Faiza, A., and Fatma E.M. (2015): Effect of *Spirulina platensis* and *Lactobacillus rhamnosus* on growth and biochemical performance of Nile Tilapia (*Oreochromis niloticus*) fingerlings. *Int.J.Curr.Microbiol.App.Sci* (2015) 4(4): 747-763.

Zhao, M., Xie, S., Zhu, X., Yang, Y., Gan, N., and Song L. (2006): Effect of dietary cyanobacteria on growth and accumulation of microcystins in Nile tilapia (*Oreochromis niloticus*). Aquaculture, 261: 960–966.

الملخص العربي

تقييم استخدام الطحلب الأخضر المزرق كإضافات أعلاف علي معدلات النمو ومكونات الدم البيوكميائيه والتركيب البكتيري لأمعاء أسماك البلطي النيلي

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يهدف البحث لدراسة تأثير أضافه طحلب *Spirulina platensis* إلي عليقه أسماك البلطي النيلي وذلك علي معدلات النمو وبعض مكونات الدم البيوكميائية وتركيب الأسماك بعد تجفيفها. وقد استمرت هذه الدراسة لمده ثلاث شهور وتم إجرائها بمعمل الدراسات العليا – قسم أمراض الأسماك ورعايتها – جامعه المنصورة. استخدم في هذه التجربة عدد(٦) أحواض زجاجيه بكل حوض زجاجي تم وضع عدد (١٥) سمكه متوسط الحجم (٢١,٤٤ ± ٠,٣١) تم تقسيمها عشوائيا إلي مجموعتان متساويتان بكل مجموعه عدد (٣) حوض زجاجي. تم تحضير مخلوط علف مركز أساسي يتكون من ٩,٥% وجبة سمك – ١٣% غلو تين – ٢٥% فول صويا – ١٥ % نخاله القمح – ١٥% نخاله الأرز – ١٨% أزره صفراء - ٢% زيت عباد الشمس – ٢% مخلوط الفيتامين والمعادن. وقد تم تحضير عدد (٢|) عليقه تجريبية العليقة الأولي (مجموعه المقارنة) هي عبارة عن مخلوط العلف المركز الأساسي بدون أي إضافات والعليقة الثانية عبارة عن العلف المركز الأساسي مع إضافة الطحلب المجفف بنسبه (٠,٦مجم / كجم علف مركز).وقد تم قياس معدلات النمو وقياس مكونات الدم البيوكميائية ومكونات الجسم بعد التجفيف.

وكانت أهم النتائج المتحصل عليها كالتالي :-

١- أظهرت النتائج أن مقارنه بالمجموعة المقارنة إضافة الطحلب بهذه النسبة (٠,٦ جم / كم) لم تؤثر في وزن الجسم ومعدلات النمو ومعامل التحول الغذائي ومعدل النمو المحدد. ولكن معدل المأكول لم يتأثر في الشهرين الأول و الثاني ولكنه كان أقل معنويا من المجموعة المقارنة في الشهر الثالث. كما أيضا معدل البروتين المأكول معنويا بإضافة الطحلب في الشهر الثالث بالمقارنة بالمجموعة المقارنة.

٢- أظهرت نتائج تحليل الأسماك بعد تجفيفها أن نسبه البروتين زاد زيادة معنوية بالمقارنة بالمجموعة المقارنة.

٣- أظهرت نتائج تحليل بعض مكونات الدم (السيرم) مثل lysozyme والبروتين الكلي والجلوبيولين زاد زيادة معنوية بالمقارنة بالمجموعة المقارنة.

من النتائج السابقة نستنج أنه يمكن استخدام الطحلب الأخضر المزرق كإضافات أعلاف في علائق أسماك البلطي النيلي حيث أنها لا تؤثر معنويا علي معدلات النمو ولكنها تساعد علي تحسين مناعة الأسماك وقدرتها علي التغلب علي الأجسام المرضية (البكتريا المسببة للأمراض) ومكونات الدم الهامة والتي تنعكس بالإيجاب علي الحالة الصحية والإنتاجية للأسماك البلطي النيلي بصفه عامة.