

SPRAY-DRIED *SPIRULINA* (*SPIRULINA PLATENSIS*) AS GROWTH PROMOTER FOR NILE TILAPIA (*OREOCHROMIS NILOTICUS*) UNDER LAB AND FIELD SCALE CONDITION

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SUMMARY

The motivation behind this study is to determine carefully the best supplemental level of spray dried *Spirulina platensis* as growth promoter for Nile tilapia (*Oreochromis niloticus*) and its effect on their performance, feed utilization, chemical body composition and gut health. Two successive experiments were conducted (Field and Lab scale). The Field – scale was conducted with a hapa-in-pond system with diets containing four levels of *Spirulina platensis* (0, 0.03, 0.05 and 0.07%) to determine the best level to supplement in tilapia diet. The Lab-scale experiment consisted of four treatments diets (Control one without any supplementation and three diets comparing between the best level of *Spirulina* obtained from the previous experiment and two standard commercial growth promoter with standard level of each one (Probiotic or prebiotic) (0.2% Biogen[®], 0.1 % Garlin Plus[®] /kg diet) respectively. Histological sections were collected to examine the gut health condition. The results of Field scale experiment showed that the dietary inclusion of 0.03 % *Spirulina* provided the best growth performance, feed utilization, and crude protein deposition than the other supplemented levels. On Lab scale side, *spirulina* at 0.03% conducted the same effect as commercial growth promoter (probiotic and prebiotic) in the same context, the intestine histological sections showed an increase of intestinal villi length in Nile tilapia. It could be concluded that *Spirulina* has a positive effect on gut conditions and that despite green water conditions contains natural cyanobacteria, the inclusion of 0.03 % of spray-dried *Spirulina* into tilapia diet positively affected growth performance and improve gut health.

Keywords: *Spirulina*, growth promoter, Nile tilapia, growth performance

INTRODUCTION

Global tilapia aquaculture has been expanding at an exceptional rate during the past few decades (FAO 2016). The global production of farmed tilapia has increased from 383,654 tons in 1990 representing 4.5% of total farmed fish production to 4,507,002 tons in 2012, representing 10.2% of farmed fish production, with an average annual growth of 13.5% (FAO 2014). This rapid growth of tilapia production requires more costly input, with an increasing dependence on formulated feeds (El-Sayed, 2006). Therefore, finding novel functional feed supplements have been a major challenge facing tilapia feed industry.

Spirulina (*Spirulina platensis*) is microalga of the class cyanophyceae. It is rich in proteins, Vitamins, essential amino acids, minerals, carotenoids and essential fatty acids (Jaime- Ceballos *et al.*, 2006; Deng and Chow 2010). The effect of *Spirulina* on growth parameters has been documented for several fish species as a partial supplementation or complete replacement for protein in aqua feeds (Sultana *et al.*, 2012 and Teimouriet *al.*, 2013a,b), as growth promoter (Abdel-Tawwab and Ahmed 2009) also provides as a source of carotenoids (Regunathan and Wesley 2006 and Teimouri *et al.*, 2013b). *Spirulina* has also been used as a feed attractant (Silva-Neto *et al.*, 2012). Further, *Spirulina* is used in wastewater treatment (Kamilya *et al.*, 2006). Additionally, *Spirulina* has been speculated to have a positive effect on the immune system. *Spirulina* may potentiate the immune system leading to preventing to cancer development and viral infection (Hirahashi *et al.*, 2002 and Ibrahim and Ibrahim; 2014).

Different forms of *Spirulina*, fresh or dried have been used as a supplement in animal's diets. *Spirulina* preparation and drying methods greatly affect its nutritional value. The spray dried method is the common method of producing *Spirulina*. Lin., (1985) found that bacterial and chemical content of spray and freeze dried *Spirulina* were found to be superior to conventional drying methods.

Despite previous results of many trials using dietary *Spirulina* in feed rations of many animal species that showed improving productivity, health, and product quality, their findings are contradictory. Subsequently, more investigation is required to assess *Spirulina* potentials because of its inconclusive results as an animal feed supplement (Holman and Malau-Aduli 2013)

Primary objectives of this study were to investigate the effect of supplementing tilapia diet with spray-dried *Spirulina* (*Spirulina platensis*) as growth promoter. Two successive experiments were conducted (Field and Lab scale). The field scale experiment conducted to investigate the optimum level of *Spirulina platensis* to be supplemented in tilapia diet as growth promoter. The lab scale experiment compare among the best level of *Spirulina* determined from field experiment and two commercial growth promoter (probiotic, prebiotic) and study to what extent it may affect tilapia performance and gut health.

MATERIALS AND METHODS

Experimental diets

Spray-dried *Spirulina* were obtained from a commercial company (IGV Food tech, Germany). The composition and chemical analysis of basal diet is given in Table (1). The dietary ingredients were finely ground, weighed, blended and pelleted using a meat mincer. Diets were dried in the oven at 60 °C for 24 hours, and crushed for adjusting feed particle size and stored at - 4°C.

Table (1): Composition and chemical analysis of the basal diet used in the experiment.

Ingredient	%
Wheat tag	30
Soya bean meal	20
Fish meal	20
Glutein meal	15
Corn grain	4
Corn oil	4
Di-ca-phosphate	2
Vitamins & minerals Premix ¹	3
Caco ₃	2
Total	100
<i>Chemical analysis %</i>	
Dry Matter	90.07
Crude protein	35.00
Ether Extract	8.00
Total carbohydrates	36.07
Ash	8.00
Gross energy Kcal/Kg diet	4356

¹Contains(Kg⁻¹): vitamin A, 3,333,333 IU; vitamin D₃, 833.333 IU; vitamin E, 3,333 mg; vitamin K, 333 mg; vitamin B₁, 333.3 mg; vitamin B₂, 1,667 mg; vitamin B₆, 500 mg; vitamin B₁₂, 3.33 mg; niacin, 10,000 mg; pantothenic acid, 3,333.3 mg; folic acid, 333.3 mg; biotin, 16.7 mg; iodine, 100 mg; iron, 10,000 mg; manganese, 20,000 mg; copper, 1,333 mg; cobalt, 33.3 mg; selenium, 33.3 mg; zinc, 16,667 mg; and calcium carbonate, 1,000 mg.

Field-scale experiment

This experiment lasted for 92 days in 12 hapas (2 m³) with 0.5 meters deep in the pond water. The experimental hapas were suspended in an earthen pond 70 x 30 m (2100 m²) at a commercial farm of mono-sex tilapia located in Kafer El-Sheikh Governorate, Egypt. They were supplied with brackish water (2500 ppm) under the condition of hapa – in- pond system.

Mono-sex Nile tilapia fry of 0.25 g weight were distributed randomly to the twelve experimental hapas at fixed total stocking density of 100 fish / hapa (equivalent to 50 fish / m³). The twelve hapas were divided to provide three hapas (replicates) for the control group fed basal diet and three hapas (replicates) for each diet supplemented with different levels of *Spirulina* (0.03, 0.05, and 0.07%).

The four groups of fish were manually fed. The feeding rate was 10% (30 days), 8% (30 days), and 5% (32 days) of their live body weight per day, divided into four feeding times at 9:00 am, 12:00 pm, 2:00 pm, and 4:00 pm. Every two weeks fish from each hapa were sampled and weighed to the determined average weight of fish and the ration was adjusted according to the new weight.

Water Quality

Daily water temperature and dissolved oxygen (DO) readings were recorded throughout the duration of the study at 6.00 am and 2.00 pm using water proof DO meter (Model HI 9146-04, Hanna Instrument Co.), while water pH was measured weekly at 2:00 pm using digital pH meter, Water alkalinity and total ammonia nitrogen (TAN mg/ L) were determined biweekly (Hanna Instrument test kits Co.). Water salinity (mg/L) was determined using a digital conductivity meter (model 4075, Jenway). The experimental water criteria were determined according to APHA (1999) Table (2).

Lab-scale experiment

A total of 300 mono sex Nile Tilapia (*Oreochromis niloticus*) fingerlings were obtained from a private tilapia hatchery (Kafr El-Sheikh, Egypt). Fish had an average initial weight of 2.8 g were held in two glass aquaria with water capacity of 90 L for two weeks acclimatization. Experiments were carried out in 12 experimental glass aquaria each with a water volume of 40 liters.

The experiment consisted of four treatments in triplicate the control one with no supplementation and three treatments to compare the best level of *Spirulina* determined from Field experiment (0.03%) in the presence of dietary supplementation with two commercial growth promoter in their recommended level probiotic or prebiotic (2gBiogen[®], 1gGarlin Plus[®]/kg diet) respectively. Each replicate contained 20 fry. Fish were fed at 5% of their body weight for two weeks then reduced to 3%. Feed were delivered twice daily at 9 am and 3 pm. The experiment lasted for 77 days, where fish were weighed every fourteen days and feed amounts were adjusted accordingly. Water temperature ranged between 25 and 27 °C while, the pH value was 7.5 supporting the alkalinity needed for tilapia. Aquaria were supplemented with air pump.

Histological sections

At the end of the lab-scale feeding trail, to examine the effect of *Spirulina* on gut health, Histological sections from different organs (Intestine, Liver, and kidney) of Nile tilapia were collected and frozen for examination in the histology lab (Faculty of Veterinary, Cairo University) The sections were fixed in 10% neutral buffer formalin, processed by paraffin embedding method and stained with Hematoxylin and Eosin stain (Bancroft *et al.*, 1996).

Chemical analysis of diets and fish

Samples of ingredients, diets, and fish were taken at the beginning and end of the experiments and were analyzed for moisture, crude protein (N x 6.25) lipid and ash content was analyzed according to AOAC (2006).

At the end of both field and lab scale experiments the growth performance parameters, feed utilization, and chemical body composition analysis were determined.

Statistical analysis

Statistical package SPSS version 16 (2007) was used for conducting statistical analyze. Means for significant differences were tested using Duncan's Multiple Range test (1955).

RESULTS AND DISCUSSION

The average values of water quality parameters measured throughout the experimental period are summarized in Table (2). Concerning the parameters measured, it was noted that, all values of ponds were closely suitable for the normal growth of warm water fish. These results are in agreement with those reported by Tahoun (2007).

Field scale experiment

Average values of Initial Weight (IW), Final Weight (FW), Weight Gain (WG), Average Daily Gain (ADG), Specific Growth Rate (SGR) and Survival Rate (SR) of Nile tilapia fry fed diets supplemented with different supplemental levels of *Spirulina* as growth promoter at levels (0%, 0.03%, 0.05% and 0.07%) are illustrated in Table (3). The initial weight was similar in all treatments group with no

significant differences ($p>0.05$). Interestingly, there were significant differences ($p<0.05$) among all treatments in all growth performance parameters declared superiority of the treatment supplemented with *Spirulina* at 0.03% in final weight, WG, ADG and SGR (44.56g, 44.31g, 0.48g and 5.62) respectively compared with the other treatments followed by control and 0.05%, the worst treatment was noticed in group fed on high level of supplementation 0.07% an all pervious growth parameters (39.43g, 39.18g, 0.43g, and 5.48) respectively. Additionally, there was no significant difference noticed in survival rate in all treatments.

Table (2): Water quality criteria of the pond's water (Field scale).

Month	Temp. (°C)	pH	Dissolved oxygen		TAN ¹ (mg/L)	Total Alkalinity ² (mg/L)
			am	pm		
August	28.9	7.95	2.3	6.50	0.18	169.0
September,	27.4	7.80	2.5	6.70	0.17	187.0
October,	25.1	7.90	2.7	6.90	0.16	165.0
November,	24.7	7.65	2.4	6.50	0.15	145.0

¹Where TAN means (total ammonia nitrogen).

²Expressed as CaCO_3 (mg/L).

Table (3): Effect of different levels of spray dried *Spirulina* on growth performance and survival rates (Field scale).

Treatments	initial weight (g)	Final weight (g)	Weight gain ¹ (g)	Average daily gain g/day	Specific growth rate ²	Survival Rate %
Control	0.25±0.003	41.12 ^b ±0.325	40.86 ^b ±0.326	0.44 ^b ±0.003	5.53 ^b ±0.007	98±0.882
0.03%	0.25±0.003	44.56 ^a ±0.795	44.31 ^a ±0.798	0.48 ^a ±0.010	5.62 ^a ±0.024	99±0.333
0.05%	0.25±0.00	40.50 ^{bc} ±0.158	40.24 ^{bc} ±0.159	0.44 ^b ±0.000	5.52 ^{bc} ±0.007	99±0.577
0.07%	0.25±0.003	39.43 ^c ±0.072	39.18 ^c ±0.072	0.43 ^b ±0.003	5.48 ^c ±0.000	99±0.333

Means followed by different letters in each column are significantly ($P<0.05$) different

¹Weight gain (WG) = final weight – initial weight.

²Specific growth rate (SGR) = $100 (\ln W_2 - \ln W_1) / T$

Where W_1 and W_2 are the initial and final weight, respectively, \ln represent Natural logarithm and T is the number of days in the feeding period.

Feed utilization parameters expressed as Feed intake (FI), Feed conversion rate (FCR), Protein efficiency ratio (PER), Protein productive value (PPV) and Energy utilization (EU) were illustrated in Table (4). The results clearly demonstrated enhancement in (FCR) significantly ($p<0.05$) in treatment supplemented with 0.03% *Spirulina* with best FCR values (1.68) compared with the other supplemented levels although group 0.03% recorded the lowest feed intake (74.26g) which reflected on enhancement on the pervious growth performance parameters.

Table (4): Effect of different levels of spray dried *Spirulina* on feed utilization parameters of Nile tilapia (Field scale).

Treatments	Feed intake (g)	Feed conversion ratio ³	Protein efficiency ratio ⁴	Protein productive value(%) ⁵	Energy utilization(%) ⁶
Control	77.06 ^{ab} ±0.582	1.88 ^b ±0.027	1.51 ^b ±0.024	33.05 ^b ±0.785	21.17 ^b ±0.631
0.03%	74.26 ^b ±1.733	1.68 ^a ±0.064	1.71 ^a ±0.067	39.12 ^a ±2.008	24.91 ^a ±1.299
0.05%	77.95 ^a ±0.522	1.94 ^b ±0.006	1.47 ^b ±0.003	32.57 ^b ±0.448	20.71 ^b ±0.233
0.07%	78.00 ^a ±0.322	1.99 ^b ±0.006	1.44 ^b ±0.003	31.89 ^b ±0.393	20.05 ^b ±0.238

³Feed conversion ratio (FCR) = dry feed intake (g) / fish live weight gain (g).

⁴Protein efficiency ratio (PER) = 100 (weight gain (g) / protein intake (g))

⁵Protein productive value (PPV) = 100 (protein gain (g) / protein fed (g)).

⁶Energy Retention (ER) = Retained energy in carcass (Kcal)/energy intake (Kcal) ×100.

Results indicated significant differences (p<0.05) in PER, PPV and EU among fish groups. The results showed superiority of the group supplemented with 0.03% *Spirulina* at all the pervious parameters (1.71,39.12 and 24.91%) respectively than the other groups which not significantly differ among each other (p>0.05) in the utilization parameters.

Results of body composition of Nile tilapia are illustrated in Table (5). No significant differences (p<0.05) was noticed among all treatments in Dry matter (DM), Ether extract (EE) or Ash content. On the other hand, there were significant differences (p<0.05) among treatments in Crude protein (CP) indicating that the highest protein content found in treatment supplemented with 0.03% *Spirulina* (69.63%) . However, no significant differences noticed among the other treatments in CP content (control,0.05 and 0.07%) *Spirulina*.

Results of the field scale declared that the best level supplemented in Tilapia diet is 0.03% spray dried *Spirulina* as growth promoter than the other supplemented levels (0, 0.05 and 0.07%) in growth performance, feed utilization and body composition.

Table (5): Effect of different levels of spray dried *Spirulina* on body composition of Nile tilapia (Field scale).

Treatments	Dry matter	% on dry matter basis		
		Crude protein	Ether extract	Ash
Control	31.72±0.194	68.47 ^b ±0.260	15.63±0.467	13.20±0.351
0.03%	32.28±0.322	69.63 ^a ±0.318	16.40±0.231	13.40±0.115
0.05%	31.96±0.345	67.83 ^b ±0.441	15.90±0.058	13.33±0.088
0.07%	32.03±0.186	68.10 ^b ±0.305	15.30±0.416	13.50±0.379

Means followed by different letters in each column are significantly (P<0.05) different.

Lab scale experiment

Average values of initial weight(IW),final weight(FW), Average weight gain(WG), Average daily gain(ADG), Specific growth rate(SGR) and Survival rate(SR) of Nile tilapia fingerlings fed diets supplemented with best level of *Spirulina* determined from the previous experiment (Field scale) 0.03% and two commercial growth promoters probiotic (Biogen[®]), Prebiotic(Garlin Plus[®]) are illustrated in Table (6) . The initial weight was nearly similar in all treatments group with no significant differences (p>0.05). The results declared superiority of *Spirulina* 0.03%, probiotic and prebiotic than the control treatment (p<0.05) in all growth performance parameters .And declared no significant differences among these three treatments (0.03% *Spirulina*, probiotic or prebiotic). The highest survival rate (SR) noticed in treatment 0.03% *Spirulina* (99%), followed by probiotic (97%) with no significant difference, then prebiotic (95%), the lowest SR was reordered in the control (92%).

Table (6): Effect of different levels of spray dried *Spirullina*, probiotic and prebiotic on growth performance and survival rates of Nile tilapia (Lab scale).

Treatment	Initial weight (g)	Final weight (g)	Average weight gain(g)	Average daily gain (g/ day)	Specific growth rate (%)	Survival rate (%)
Control	2.73±0.02	11.43 ^b ±0.36	8.69 ^b ±0.38	0.11 ^b ±0.020	3.04 ^b ±0.05	92.00 ^c ±2.45
0.03% (<i>Spirulina</i>)	2.62±0.18	13.92 ^a ±0.49	11.30 ^a ±0.58	0.15 ^a ±0.015	3.32 ^a ±0.05	99.00 ^a ± 1.67
Probiotic	2.78±0.02	13.94 ^a ±0.69	11.15 ^a ±0.68	0.14 ^a ±0.009	3.32 ^a ±0.07	97.00 ^{ab} ± 1.67
Prebiotic	2.77±0.03	13.11 ^a ±0.19	10.34 ^a ±0.22	0.13 ^a ± 0.000	3.24 ^a ±0.02	95.00 ^b ±2.5

Means followed by different letters in each column are significantly (P<0.05) different

Feed utilization parameters expressed as Feed intake (FI), Feed conversion ratio(FCR), Protein efficiency ratio (PER), Protein productive value (PPV) and Energy utilization (EU) were illustrated in Table (7).Meanwhile , the lowest feed intake recorded for 0.03% *Spirulina* (24.45g) but it have the best FCR (2.18) followed by probiotic (2.25) and prebiotic (2.44) with no significant differences (p>0.05) which reflected with improvement in growth performance parameters declared previously in the previous

table mention to superiority of the treatments supplemented with *Spirulina*, probiotic, prebiotic than the control one . Similarly, the same trend was observed in the other feed utilization parameters PER, PPV and Energy utilization.

Table (7): Effect of different levels of spray dried *Spirulina*, Probiotic and Prebiotic on protein and feed utilization of Nile tilapia (Lab scale).

Treatment	Feed Intake (g)	Feed conversion ratio	Protein efficiency ratio	Protein productive value (%)	Energy utilization (%)
Control	22.92 ^d ±0.01	2.65 ^a ±0.12	1.08 ^b ±0.05	23.71 ^b ±0.85	17.49 ^b ±0.62
0.03% <i>Spirulina</i>	24.45 ^c ±0.06	2.18 ^b ±0.12	1.32 ^a ±0.07	28.60 ^a ±1.19	20.75 ^a ±0.87
Probiotic	24.91 ^b ±0.01	2.25 ^b ±0.13	1.28 ^{ab} ±0.08	30.16 ^a ±1.62	21.39 ^a ±1.16
Prebiotic	25.18 ^a ±0.02	2.44 ^{ab} ±0.06	1.17 ^{ab} ±0.03	26.60 ^{ab} ±0.48	19.08 ^{ab} ±0.34

Means followed by different letters in each column are significantly ($P < 0.05$) different

The data of body composition are given in Table (8). *Spirulina* incorporation in tilapia diet did not affect body composition except for protein content, where the best value was recognized for fish fed on probiotic 65.50% without a significant difference with fish fed prebiotic(64.75) or *Spirulina* (62.45) while the lowest value ($p < 0.05$) was recorded for the control at (61.52).

Table (8): Effect of different levels of spray dried *Spirulina*, Probiotic and Prebiotic on body composition of Nile tilapia (Lab scale).

Treatments	Dry matter	% on dry matter basis		
		Crude protein	Ether Extract	Ash
Control	30.49±0.865	61.52 ^b ±2.050	23.06±0.630	11.88±0.365
0.03% <i>Spirulina</i>	30.81±1.160	62.45 ^{ab} ±1.260	22.48±0.025	11.98±0.395
Probiotic	31.50±0.229	65.50 ^a ±0.764	22.30±0.534	12.13±0.186
Prebiotic	30.50±1.500	64.75 ^{ab} ±0.250	22.68±0.070	11.55±0.050

Means followed by different letters in each column are significantly ($P < 0.05$) different

Based on histological sections, control group showed normal villi height Fig.(1), and indicated no abnormality in intestine and liver sections, except for kidney sector that showed hemorrhage in intestinal tissue and hydropic degeneration in the epithelial lining the renal tubules Fig.(2,3). Fish fed on 0.03% and probiotic showed an increase in villi length Fig. (4, 5) Liver and Kidney sections for fish fed on *Spirulina* showed abnormality in the form of hemorrhages, necrosis and granuloma Fig.(6, 7).Histological sectors confirm the beneficial effect of *Spirulina* on the gut condition where intestine of fish fed on *Spirulina* indicated an increase in villi height.

Results from two experiment showed that the best supplemented level of *Spirulina platensis* as growth promoter was 0.03% which affect positively on Tilapia performance and gut health ,this effect not differ than the effect of other known commercial growth promoters(probiotic and prebiotic).

Our results are in agreements with the results of (Abdel-Tawwab and Ahmed 2009) they suggested the positive effect of *Spirulina* as a growth promoter in Tilapia diet although they examined higher dietary inclusion than our diets they found that the optimum growth and feed utilization for Nile tilapia were obtained when fed on 0.5% live *Spirulina* . This differences between our results may be due to the type of *Spirulina* used (*Arthrospira platensis*) and our species. Additionally the difference in the nature of *Spirulina* used (live and spray dried).

Ekpo and Bender (1989) found that tilapia digest blue green algae more efficiently than Silver carp, which are strict phytoplankton feeders. They reported that the protein apparent digestible coefficient (ADC) in tilapia varied between 90.0 and 94.5% in diets with *Spirulina* sp. The capacity to digest microalgae protein may be due to the low pH in herbivorous fish stomach, which allows them to leach nutrients from the cell without breaking its cell wall (Horn and Messer 1992). This process would be very efficient for Nile tilapia considering that its stomach has the lowest pH level reported for a fish species, with values close to one (Ekpo and Bender 1989). In the same trend, Sarker *et al.*, (2016) found that the improved digestibility of crude protein and most essential amino acids of *Spirulina* sp. suggests that this microalgae is a good candidate as an alternative protein source in tilapia feed. The same was suggested by (Abdel-Tawwab and Ahmed, 2009), where different *Spirulina* levels positively affected protein content of tilapia body composition but no significant difference was observed for dry matter or ash content.



Fig. (1) Intestine of fish in control group showing normal structure and height of villi. H&EX100.

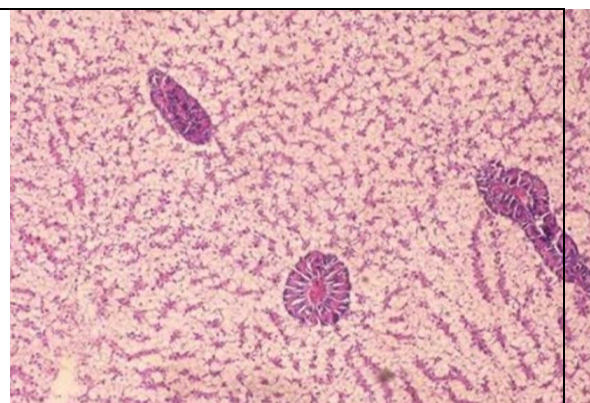


Fig. (2) Liver of fish in control group showing normal histological appearance.

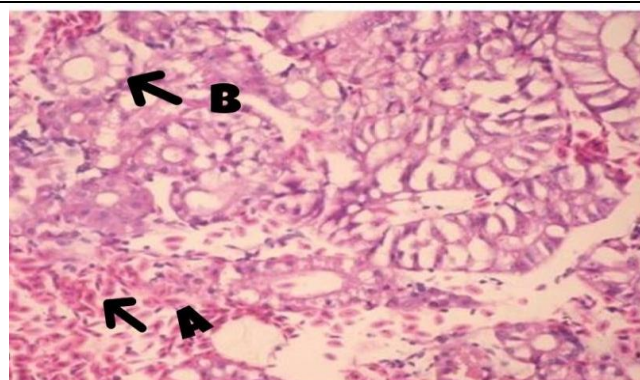


Fig. (3) Kidney of fish in control group showing (A) hemorrhage in interstitial tissue and (B) hydropic degeneration in the epithelial lining the renal tubules. H&EX 400.



Fig. (4) Intestine of fish treated with 0.03% of *Spirulina* showing increase in length of intestinal villi. H&EX 100.

In contrast with our observation, carp fed different diets with the elevated level of *Spirulina* showed no difference in protein content (Nandeesh *et al.*, 1998). Improvement in carcass protein content could be explained by the gut histology, where the intestinal section of fish fed on *Spirulina* showed an increase in villi length compared to control diet. Improving the gut condition may lead to more effective absorption of protein.

Obviously the improvement in growth performance, feed utilization and protein content on the fish fed diet 0.03% *Spirulina* have approximately equal performance with other well documented studies of commercial growth promoters probiotic (Biogen[®]) and prebiotic (Garlin Plus[®]).

The present results were in agreement with the results obtained by many authors. Ali *et al.*, 2010; Agouz and Anwer (2011) pointed out to the improvement of digestion and metabolism in the fish body due to the presence of the bacillus in the probiotic (Biogen[®]), moreover the prevention of pathogenic bacteria colonies in fish gut. Renuka *et al.* (2013) suggested that the incorporation of probiotic in common carp diets stimulated fish growth and digestion as micro biota colonization enzymes that hydrolyze complex molecules, facilitate better digestion and absorption of macronucleus resulting in higher protein and energy deposition in the body tissues. In these aspects, Faramarzi *et al.*, (2011) was in accordance with the results obtained in the present work, where they found that the addition of probiotics (*Bacillus subtilis* c-3102 spores) in Common carp fry diets improved fish growth and mitigated the effects of stress factors. In this particular, diets supplemented mix of Lactobacillus SP. spores resulted in improving growth performance of Striped mullet (*Mugil cephalus*) significantly than those fed the control basal diets (El-Tawil *et al.*, 2012)

Mohapatra *et al.* (2012) who found that incorporation of live probiotic microorganisms (*Lactobacillus lactis* and *Bacillus subtilis*) resulted in maximum growth performance in rohu (*Labeo rohita*) fingerlings in comparison with some combinations of inactivated probiotics.

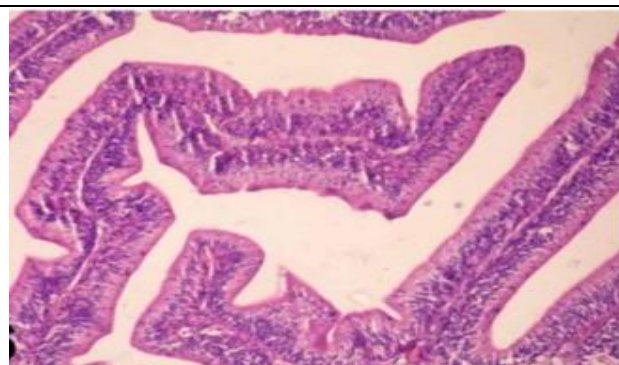


Fig. (5) Intestine of fish treated with probiotic showing increase in length of villus height. H&EX200

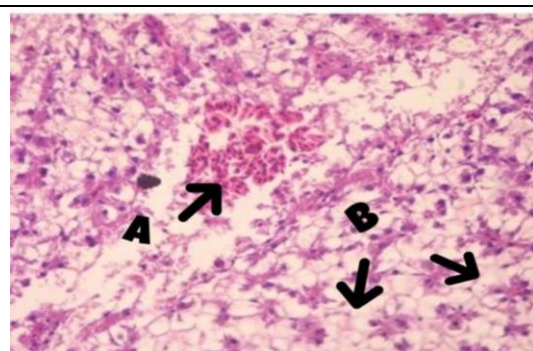


Fig. (6) Liver of fish treated with 0.03% of *Spirulina* showing (A) focal hemorrhagic areas dispersed the hepatocytes and (B) individual necrosis of hepatocytes. H&EX 200.

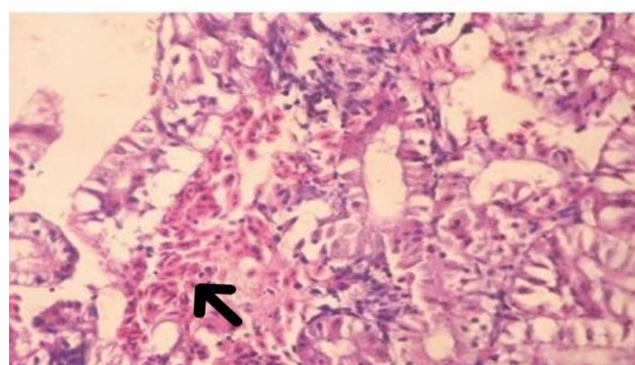


Fig. (7) Kidney of fish treated with 0.03% of *Spirulina* showing hemorrhagic area in interstitial tissues. H&EX 200.

Other similar results were also observed for Nile tilapia (Lara-Flores *et al.*, 2003), *Cyprinus carpio* (Ramakrishnan *et al.*, 2008) they found that the incorporation of *Spirulina* and probiotics in Common carp diets improves growth performance and total heterotrophic microbial load. The *Spirulina* diets were most effective in stimulating fish growth. Additionally, the fish fed diet supplemented with prebiotic showed improvement it was suggested that the improvement in growth parameters occurred with the prebiotic diets may be due to the fermentation of prebiotics in colon which promote the growth of the bacterial populations associated with the healthy well-functioning colon. Beneficial types of colonic bacteria have the ability of oligosaccharides fermentation which is not used effectively by potentially pathogenic bacteria species (Yosefian and Amiri 2009). As in the case of probiotics, the positive effect of prebiotics on growth was also found in different fish species such as Atlantic salmon, (Refstie *et al.*, 2006) ,Rainbow trout (Grisdale-Helland*et al.*,2008) Rainbow trout, Hybrid tilapia (Genc and Yilmaz 2007 Yilmaz *et al.*, 2007) .

From all pervious discussion and go parallel with the scope of Silva –Nato *et al.* (2012) It would be possible to achieve better growth performance results if higher levels of *Spirulina* had been used in the present work . But, it was not our purpose to test *Spirulina* as a source of macronutrients, such as amino acids or fatty acids or even as protein source in fish diets. Our aim was to test *Spirulina* as a feed additive (growth promoter), included at very low dietary levels. This question needs to be heighted because the price of *Spirulina* is very high making its use not economically feasible to high dietary inclusion levels.

CONCLUSION

Despite the green water contained natural cyanobacteria, the inclusion of spray dried *Spirulina* at 0.03% in Nile tilapia diet improved growth performance, feed utilization and crude protein content under field conditions. At lab scale, it may have a positive effect on gut condition similar to probiotics and prebiotics.

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استخدام طحلب الاسبيرولينا المجفف بطريقة الرش كمحسن نمو لاسماك البلطى النيلى تحت الظروف المعملية والحقلية

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تهدف هذه الدراسة الى التعرف على المستوى الامثل من اضافة طحلب الاسبيرولينا المجفف بطريقة الرش كمحسن نمو فى علائق البلطى النيلى و كذلك مقارنة المستوى الامثل من الاضافة بنوعين تجاريين من محسنات النمو وتأثير ذلك على مقاييس النمو والاستفادة الغذائية وصحة الامعاء لاسماك البلطى النيلى.

وقد أجريت هذه التجربة على النطاق الحقلى وعلى النطاق المعملى وتكونت التجربة الحقلية من أربعة علائق غذائية للتعرف على المستوى الامثل لاضافة الاسبيرولينا (صفر، 0.03% ، 0.05% ، 0.07%) فى علائق زريعة البلطى النيلى لمدة 92 يوم فى هابوات بأحواض ارضية .

وتمت التجربة المعملية لمدة 77 يوما لمقارنة المستوى الامثل من اضافة الاسبيرولينا مع نوعين من المنشطات الحيوية (البروبيوتك والبريبوتك) كلا مضاف بالنسبة الموصى بها (بيوجين 0.2% و جارلين بلس 0.1%) بالتتابع وفى نهاية التجربة تم أخذ قطاعات هستولوجية من الاعضاء المختلفة (الامعاء والكبد والكلى) وذلك للتعرف على حالة الجهاز الهضمى لاسماك .

أوضحت النتائج على مستوى الحقل أن أفضل نسبة من طحلب الاسبيرولينا كمحسن نمو كانت 0.03% /كجم علف حيث أعطى أفضل معدلات نمو واستفادة غذائية و أفضل نسبة بروتين خام فى جسم الاسماك .

وأظهرت النتائج على مستوى المعمل أن طحلب الاسبيرولينا قد أظهر أداء انتاجى ومعدل استفادة غذائية وزيادة فى مستوى بروتين الجسم كما أظهرته المعاملات المغذاه على البروبيوتك والبريبوتك مقارنة بالمعاملة الكونترول التى لم يضاف لها أي اضافات و قد أظهرت القطاعات هستولوجية للامعاء زيادة فى طول الخملات فى الامعاء فى العلائق المضاف لها الاسبيرولينا والبروبيوتك .

التوصية: أن طحلب الاسبيرولينا له تأثير ايجابى على حالة الامعاء وان اضافة نسبة 0.03% من الطحلب المجفف بطريقة الرش يحسن أداء النمو لاسماك البلطى النيلى على المستوى التطبيقى.