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Effect of various levels of Spirulina (*Arthrospira platensis*) as feed supplement on growth performance, feed utilization , immune response and hematology of the Nile tilapia (*Oreochromis niloticus*) fingerlings.

Ahmed M. Al-Zayat
Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt.

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ABSTRACT

The objective of this study was to evaluate the effect of graded levels (0.0 , 2.5, 5.0 and 7.5g /kg diet) of the dietary blue-green microalgae (*Spirulina*) *Arthrospira platensis* as feed supplement to assess growth performance , nutrient utilization, immune response , some hematological parameters, and proximate composition of the Nile tilapia (*O. niloticus*) **.** A completely randomized experimental design was developed with 4 treatments and two replicates . Fish (80 all-male Nile tilapia fingerlings) ,with an average initial weight of 6±0.20 g , were randomly distributed into 8 glass aquaria (112 liter) and each aquarium holding 10 fish and randomly assigned to one of two replicates of the diets and offered feed to satiation to fingerlings O. niloticus. Fish were fed a balanced diet of 30% protein along the period of the experiment. The treatments were Diet 1 (Control) without *Spirulina*, Diet 2 (2.5g *Spirulina* /kg diet), Diet 3 (5g *Spirulina* /kg diet) and Diet 4 (7.5 g *Spirulina* /kg diet). The results indicate that, the fish groups received *Spirulina* supplemental diets revealed significant improvement in growth parameters (body weight gain, feed conversion ratio and protein efficiency ratio). Since the diets contained microalga *Spirulina* at a level (7.5 gram/kg diet) showed the highest values of growth parameters and protein utilization. Carcass composition of the experimental fish was relatively affected by different dietary treatments. This study suggested that *Spirulina* at a level of 7.5 gram/kg can be used in the feed of Nile tilapia without negative effect on growth parameters, feed utilization and blood parameters.

INTRODUCTION

Tilapia is one of the most important groups of fish for aquaculture with annual production exceeds two million metric tons. They have been cultured for quite a long time ago. However, their aquaculture production has been developed remarkably during the last few decades due to the increased level of intensification and cultured area (Trinhu, 2008). In many aquaculture operations today, feed accounts, more than half of the variable operating cost (NRC, 1993). Therefore, the potential use of unconventional food stuffs such as algae, for substitution the high cost foodstuffs such as fish meal is very important. Algae have attracted the attention as a possible alternative protein source for cultured fish, particularly in tropical and subtropical developing countries, where algae production rates are high and they have higher protein, vitamins and essential fatty acid contents. Algae have a high content of protein (50-80%), low fiber (10%) and high content of threonine, lucien, phenyl alanine, tyrosine and valise (Brune, 1980). Dawah *et al.*, (2002) found that five amino acids (aspartic acid, serine, alanine, leucine and lysine) were collectively responsible for 50% or more of the total dry matter content and algae proteins like other single cell protein are deficient in sulfur amino acids. Aquaculture developed as an alternative way to produce fish without decreasing wild fish stocks. Since the 1990s aquaculture has made a significant contribution to the world's fish supply (Sumaila *et al*., 2016) where half of the seafood consumed comes from aquaculture (Fry *et al*., 2016). Nonetheless, the rapid increase of aquaculture production and its industrialization has led to a number of environmental problems due to eutrophication (Edwards, 2015) and emission of substances like hormones, antibiotics and biocides (Bergleiter and Meisch, 2015).

To sustain such high rate of increase in aquaculture production, a similar increase in the levels of fish feed production is required. This activity requires high quality feeds, which should contain not only necessary nutrients, but also complementary feed additives to keep organisms healthy, favor growth and environment friendly aquaculture. Feed accounts for more than half of the variable operating costs in many aquaculture operations today (NRC, 2011). Therefore, the potential use of unconventional feed ingredients such as to Algae, as feed input as a replacement of high cost feed stuffs such as fish meal, soybean meal and yellow corn used. Properly formulated feeds are a significant part of successful aquaculture. *Arthrospira Platensis* (*Spirulina*) organic materials consisted of protein 60-70% carbohydrate 10-20%, fat 5% and fiber 2% (Hendrickson, 1989). According to the findings of (Paulauskas and Kulpys, 2007) protein of *Arthospira Plastensis (Spirulina)* have more valuable amino acids as well as biologically active substances such as vitamins, minerals, polysaccharides. Antioxidant characteristics of cyanobacteria are based on its contents of phytohormones and enzymes. Because of its biological active substance's unique chemical composition, valuable proteins, the correct proportion of amino acids, and amounts of vitamins and minerals, the biomass of *Spirulina* algae can be successfully used in animal nutrition (Wallace, 2000). Thus, the present study was conducted to determine the effect of using graded levels of *Spirulina* on growth performance, feed utilization, and carcass composition of Nile Tilapia (*O. niloticus*).

MATERIALS AND METHODS

The present study was carried out at the fish Lab, Dept. of Animal Production, Faculty of Agriculture, Al-Azhar university. During season 2018 in order to evaluate microalga (*Spirulina*) in Nile tilapia (*O.niloticus*) diet on growth performance, survival rate, feed utilization, and carcass composition under Egyptian conditions.

Spirulina cultivation

Spirulina microalga was grown in a modified Zarrouk's medium (Ravelonandro *et al*., 2008). *Spirulina* was dried according to Fadl *et al*., (2017).

Experimental fish

The experimental fish (*Oreochromis niloticus*) was collected from a private farm in Kafr El-Sheikh governorate. The fingerlings were placed in a fiberglass tank and randomly distributed into the experimental aquaria for the adaptation to the experimental conditions until starting the experiment. Fish were fed the control diet for two weeks, during this period, healthy fish at the same weight replaced the diet one.

Experimental design of rearing fish

A group of 80 Nile Tilapia fingerlings (*O. niloticus*) fish with an average initial body weight 6 grams were randomly allotted into 8 glass aquaria (80/35/40 cm). With 10 fish in each aquarium and each treatment was applied in two aquaria. Fresh tap water was stored in fiberglass tanks for 24h under aeration for dichlorination. One third of the water in each aquarium was replaced daily and totally changed once every week after removing the wastes. 10 air stones were used for aerating the water aquaria photoperiod was adjusted to be 14h Light and 10h darkness using florescent light. Fish faces and feed residue were removed daily by siphoning.

Experimental Diets and feeding Regime

Two weeks before the beginning of the experimental trial, about the fishes were adapted to a basal diet containing 30% crude protein and consisted of fish meal, soybean meal, yellow corn, wheat bran, rice bran, gluten, sunflower oil and Vitamin mixture. Algae (*Spirulina*) was added at a level of 2.5 gram/kg diet, 5gram/kg diet and 7.5 gram/kg diet except the control as shown in table 1. The dry ingredients were grounded through a feed grinder to small particle size (0.5mm). The ingredients were weighed and mixed by a dough mixer for 20 minutes till homogeneity of the ingredients. The estimated amount of oil was gradually added (few drops gradually) and the mixing operation was continued for 20 minutes after homogenous mixture was obtained. Forty ml water per hundred g diet was slowly added to the mixture according to (Shimeino,1993). The diets were cooked in a water bath for 20 minutes, thereafter the different doses algae (2.5, 5, and 7.5 gram/kg diet) was added to the ingredients prior to palletizing. The diets were pelleted through fodder machine and pellets were dried under room temperature for 24 h before use. The chemical analysis of feed ingredients used in the experimental diets is presented in (Table 1). Composition of the mixed diets is presented in (Table 2).

Table 1: The proximate analysis of testing ingredients (% on DM basis).

Ingredients	DM	CP	EE	ASH	Fiber	NFE*	GE kcal/kg**
Gluten	92.66	69.50	2.20	1.9	2.00	24.4	510.008
Fish meal	93.58	60.52	8.43	25.5	0.60	4.95	621.031
Wheat bran	91.22	16.57	1.46	4.1	9.90	67.97	385.251
Soybean meal	92.50	48.10	1.23	6.3	7.30	37.07	422.958
Corn	90.55	7.59	1.83	0.8	2.30	87.48	418.472
Spirulina	81.82	19.82	21.82	13.56	2.80	4.20	334.192

*NFE = 100 – (CP+EE+CF+ASH).

**Gross energy was calculated by multiplication the factor 4.1, 5.6 and 9.44 kcal GE/kg DM carbohydrate, protein and fat, respectively (Jobling, 1983).

Table 2: Ingredients and composition of the experimental diet.

Ingredients	Diet1	Dite2	Diet3	Diet4
Fish meal, (FM[1])	8	8	8	8
Gluten	20	17.5	15	12.5
Soybean meal	25	25	25	25
Wheat bran	7	7	7	7
Corn	35	35	35	35
Oil	4	4	4	4
Vitamin and minerals.	1	1	1	1
Spirulina b%	0	2.5	5	7.5
Total	100	100	100	100
Chemical analysis				
Dry matter	93.31	93.42	93.92	93.82
Crude protein	30.10	30.05	30.10	30.08
Ether extract	8.44	8.24	8.27	8.21
Ash	8.24	7.99	8.30	8.49
Calculated value				
NFE	53.21	53.72	53.33	53.22
Gross energy kcal/100g	466.432	466.31	465.27	464.14
D.E (kcal/100g*)	332.90	331.85	331.59	330.73

*(Digestible energy) (kcal/100g), based on 5.0 kcal/g protein, 9.0 kcal/g Lipid, 2.0 kcal/g Carbohydrate, according to (Wee and Shu, 1989).

Growth Parameters

Average Total gain (ATG) average daily gain (ADG), specific growth rate (SGR), Feed conversion ratio (FCR), Protein efficiency ratio (PER), Protein productive value (PPV), and Survival rate (SR%) were calculated according to the following equations:

ATG (g/fish) = {Average final weight (g) – Average initial weight (g).

ADG (g/fish/day) = {ATG (g)/experimental period (d).

SGR (%/day) = {Ln Final body weight – Ln initial body weight} × 100/experimental period (d).

FCR = Feed intake (g)/live weight gain.

PER = Live weight gain (g)/protein intake (g).

PPV% = 100 {Final Fish body protein (g) – initial fish body protein (g)/crude protein intake (g).

SR = 100 {Total No. of fish at the end of the experimental period/total No. of fish at the start of the experiment.

Proximate analysis

Dry matter, crude protein, ether extract, (crude fiber) and ash contents of the tested ingredients and the whole body of fish at the beginning and at the end of the experiment were performed according (A. O. A. C., 1990).

Clinical pathological examination

hematological parameters

Hemoglobin concentration (Hb)

Blood samples from the different groups were collected from the caudal peduncle. Adequate amounts of whole blood in small plastic vials containing heparin were used for the determination of hemoglobin (Hb) by using a commercial kit (Diamond Diagnostic, Egypt).

Packed cell volume (PCV %)

PCV was estimated by the microhaematocrite method described by (Decie and Lewis, 1991).

Erythrocyte and leukocyte count

A manual method for counting using a hemocytometer counting chamber and Natt-Herrik solution was carried out according to (Stoskopf, 1993).

Biochemical parameters

Alanine aminotransferase activity (ALT) and Aspartate aminotransferase activity (AST): Colorimetric determination of ALT and AST activity was performed according to (Reitman and Frankel, 1957) using commercial kits.

Total protein: Assay of total protein was carried out by a test kit according to the method described by (Weichselbaum, 1946).

Serum albumin from all experimental groups was estimated method according to (Dumas and Biggs, 1972) using commercial kits.

Globulin: was calculated by mathematical subtraction of albumin value from total protein.

Albumin/Globulin (A/G) ratio: Albumin: Globulin ratio was calculated from data of albumin and globulin concentration.

Statistical analysis

The obtained numerical data were statistically analyzed using (SPSS, 1997) for one-way analysis of variance. When the F - test was significant, least significant difference was calculated according (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical composition of the experimental diets

Chemical composition and calculated energy of different diets are presented in (Table 2), the chemical analysis revealed that no differences were observed among all diets in DM, CP, and ASH, while there were some differences observed among different diets for EE and CF. These differences may be due to the ingredients themselves. The CP content was between 30.05 to 30.10 on DM basis. Such level was within the range suggested by (NRC, 1993; Jauncey, 1982). The calculated energy was similar in the tested diets, where the GE values ranged from 464.14 to 466.43 kcal/100g it was higher than that suggested by (NRC, 1993) in the practical diets for Tilapia. However, it was nearly similar to that used by (Hassanen *et al*., 1995; Abdel-Maksoud *et al*.. 1998).

Quality parameters of rearing water

All tested water quality criteria were suitable for rearing Nile tilapia fingerlings as cited by (Abdel-Hakim *et al*., 2002.; Abdelhamid, 2009). Since water temperature ranged between (27.5 and 28 C^{o}), pH value (7.5 and 9), and dissolved Oxygen ranged between (7 and 8.5 mg/L). Also, Abdelhamid et al., (2004) suggested that these values are suitable for rearing Nile tilapia and in the same trend, all the tested water quality (temperature, PH value, conductivity mg/L and dissolved oxygen mg/L) criteria were suitable for rearing Nile tilapia fish.

Growth performance and survival rate

At the end of the experimental period, both group D4 received *Spirulina* supplemented diet revealed significant increase in the total weight gain (TWG), average daily gain (ADG), and specific growth rate (SGR). There were significant ($P \leq 0.05$) differences among various groups of fish concerning final body weight, daily gain, specific growth rate, and survival rate of the experimented fish, being the best values in favour of D4 and D3, which seem even more better than the control and D2. These results are demonstrated in (Table 3). The best growth parameters observed with *Spirulina* supplemented diets suggested that, the addition of *Spirulina* improved feed utilization in practical terms, this mean that *Spirulina* used can decrease the amount of feed necessary for animal growth, which could result in production cost reduction, similar result have been reported by (Fadl *et al*., 2017). And these results are compatible with Xu *et al*., (2014), revealing that the growth performance and the total fish protein significantly improved in the fish feed with *Spirulina*. Also, the dietary feeding of the gibe carp by *Spirulina* lead to a significant increase in the growth. The body weight of *Spirulina* groups significantly decreased in comparison with the control. These results may be due to decrease protein in *Spirulina* (19.82%) protein. Replacement of fish meal with *Spirulina* has not affected final weight gain, specific growth rate, feed conversion ratio and survival rate of fish (Ungsethaphand *et al*., 2010). However, Abdulrahman, (2014) informed that *Spirulina platensis* powder fed to the common carp fingerlings leads to a significant improvement of the performance parameters. Meanwhile, statistical analysis of SR, showed no significant changes. These results are inagreement with Dawah *et al*., (2002) found the addition of algae in fish diets improved growth performance of Nile tilapia and disagree with Hossain *et al*., (2017) revealed that there was no significant difference in case of average daily gain. The fish and the poultry fed with *Spirulina* brought about improvement in survival rate and growth rate Hossain *et al*., 2017; Hirahashi *et al*., 2002). The survival rate of fish has not been affected by *Spirulina* diet.

Table 3: Mean's standard error of the growth performance of experiment tilapia fish as affected by the dietary treatments for 60 days.

Parameters	D1 (Control)	D2 (2.5%)	D3 (5%)	D4 (7.5%)
Initial fish weight (g)	6±0.20	6±0.20	6±0.30	6±0.30
Final fish weight (g)	28.70±0.20[c]	29.48±1.03[c]	30.65±0.15[b]	35.55±3.65[a]
Total weight gain (g)	24.70±0.20[c]	25.48±1.03[c]	26.65±0.15[b]	31.55±3.65[a]
AV. Daily gain (g)	0.42±0.01[c]	0.43±0.02[c]	0.45±0.01[c]	0.53±0.06[a]
SGR (%/ day)	1.05±0.01[c]	1.08±0.04[c]	1.12±0.01[c]	1.26±0.011[a]
No. of fish at start	10	10	10	10
No. of fish at end.	10	10	10	10
Survival ratio (SR %)	100	100	100	100

Means in the same row with different letters differ significantly (P <0.05).

Feed and protein utilization

All criteria studied and presented in Table (4) showed again that D4 was better (P≤0.05) treatments more than the control, D3 and D2 concerning FI, FCR, PER, PR%, and PPV% in Nile Tilapia then followed by D3. There were significant (P≤0.05) differences among treatments for FCR, PPV, PR%, and PER. While there were no significant differences between D2 and D3 in data of FCR, PER, and PPV. Again, D2 and D3 were the worst one, compared with other treatments. The FCR of *O. niloticus* kept on a basal diet (control) was higher than, D2, D3, groups receiving the diet supplemented with *Spirulina*, which in turn represented a positive aspect of *Spirulina* supplemented diets, The best FCR, PER, and PPV values were observed with *Spirulina* supplemented diets suggested that, The addition of *Spirulina* improved feed utilization similar results have been reported by (Dawah *et al*., 2002; Hossain *et al*., 2017). FCR and survival rate of Nile tilapia fed with *Spirulina* no significant difference in case of average daily gain. Also, Sukri et al., (2016) found that there was no significant difference in FCR in all groups, which fed on diets containing various *Spirulina* levels ranging from 0 to 10%.

Table 4: Feed intake and conversion as well as protein utilization in the experimented Nile tilapia as affected by the dietary treatments during the 60 days experimental periods.

Parameters	D1 (Control)	D2 (2.5%)	D3 (5%)	D4 (7.5%)
Feed intake (g diet/fish) (FI)	50.76±3.24[c]	52.26±0.54[b]	51.48±3.22[b]	61.26±9.59[a]
Feed conversion ratio (FCR) (g/ fish)	1.77±0.13[a]	1.78±0.08[a]	1.68±0.07 [c]	1.73±0.02[b]
Feed efficiency (FE) (g/ fish)	0.49±0.04[b]	0.49±0.03[b]	0.52±0.02[a]	0.52±0.01[a]
Protein efficiency ratio (PER) (g/fish)	1.63±0.12[b]	1.63±0.09[b]	1.73±0.07[a]	1.72±0.01[a]
Protein retention (PR %)	3.64±0.05[c]	4.42±0.27[bc]	4.54±0.13 ab[c]	5.65±0.64 [a]
Protein productive value (PPV %)	23.98±1.89[c]	28.17±2.00[b]	29.40±0.50[ab]	30.72±0.07 [a]

Means in the same row with different letters differ significantly (P <0.05).

Body composition

Values of dry matter (DM), crude protein (CP), ether extract (EE) and ASH of the fish body are summarized in (Table 5). The results of carcass composition of Nile tilapia showed that the difference were not significant (P≤0.05) in dry matter and ether extract. But crude protein differed significantly between fish groups. These results agree with the finding of (Hossain *et al*., 2017; Sukri et al., 2016), but Ungsethaphand *et al*., (2010) found that no differences were observed for moisture, ash and protein content among (D2, D3, and D4).

Table 5: Mean's ± standard errors of proximate analysis (% on the dry matter basis) of experiments fish fed on graded levels of *Spirulina*.

Proximate composition	Initial	D1 (Control)	D2 (2.5%)	D3 (5%)	D4 (7.5%)
Dry matter (DM) (%)	21.48±0.02	27.03±0.10	26.76±0.56	27.11±1.19	27.70±1.10
Crude protein (CP) (%)	64.44±0.03	53.97±0.11 b	62.94±0.11a	61.33±1.50 a	63.06±2.54 a
Ether extract (EE) (%)	9.80±0.05	20.75±0.90	15.14±1.47	16.91±1.56	15.98±3.52
Ash (%)	24.87±0.01	16.31±0.98	14.74±0.58	15.90±0.57	15.12±1.93

Means in the same row with different letters differ significantly (P <0.05).

Clinicopathological findings

Biochemical parameters

The results of protein profile and liver enzymes showed significant increases in total protein and globulin (G) and significant decrease in albumin (A), A/G ratio and liver enzymes (ALT) significant between D4 and other treatment, but (AST) no differed significantly between fish groups. These results are illustrated in (Table 6). The results could be attributed to the immune modulatory effect of *Spirulina* on the liver cell which activate the anabolic capacity of the hepatocytes produce blood protein, particularly globulin (Jessus *et al.*, 2002), and this was also supported by the results of hepatic enzyme activity, which decreased in *O. niloticus* kept on algae in comparison to the control group. These results were supported by the findings of Nayak *et al.*, 2002; Rajesh *et al.*, 2004).

Table 6: Protein profile activities of serum enzymes (ALT& AST) in *O.niloticus* groups post treatment with *Spirulina.*

Treatment	Total protein (g/100mL)	Albumin (g/100mL)	Globulin (g/100mL)	ALT (µ/l)	AST (µ/l)
D1	2.75±0.01b	0.60±0.20a	2.15±0.15b	25±0.30a	19.80±0.20
D2	3.00±0.20b	0.54±0.11ab	2.46±0.06a	16.55±0.55b	19.15±1.75
D3	2.60±0.11c	0.46±0.01b	2.14±0.16b	16.40±0.25b	19.10±0.60
D4	3.40±0.11a	0.58±0.21a	2.18±0.056b	16.80±1.30b	19.18±0.03

Means in the same row with different letters differ significantly (P <0.05).

Hematogram

The results of hematogram revealed significant increase in RBCs count, Hb value PCV % and WBCs in the two groups D4, and D3. Treated with *Spirulina* these results are presented in (Table 7). These could be attributed to the fact that, the algae used increasing the blood parameter values as a result of hemopiotic stimulation Sarma *et al.*, 2003; Rajesh *et al.*, 2006).

Table 7: Hematologram of *O.niloticus* groups post treatment with *Spirulina*.

Treatment	RBCs (x106/mm³)	Hb (g/100mL)	PCV (%)	WBCs (x106/mm³)
D1	1.45±0.05b	8.85±0.95b	25.78±1.52b	45.57±0.25a
D2	1.79±0.03a	8.15±0.95b	26.28±0.03b	37.71±0.15b
D3	1.76±0.05a	9.90±1.80a	30.75±2.79a	45.83±0.22a
D4	1.80±0.01a	9.93±1.10a	32.74±2.51a	45.59±0.26a

Means in the same row with different letters differ significantly (P <0.05).

CONCLUSION

From the previous results, it could be concluded the positive influence of adding microalga *Spirulina* g/kg to the diets of Nile tilapia on growth performance, showed positive active effects. From feed utilization date and the economical point of view, the diets supplemented with 7.5% *Spirulina* were better treated**.**

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