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Enhancing *O. niloticus* immunological and general health indicators parameters through partial substitution of soybeans with Spirulina spp. and Nannochloropsis algae

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Abstract

The present study targeted the evaluation of *Spirulina spp*. (Sp) and *Nannochloropsis* genus (Nano) as a partial substitution of soybeans on general health of *O. niloticus* through haematological analysis and histological study by seven treatments as follow: negative control (T_1), three levels of *Spirulina spp*. (Sp) 3 % Sp (T^2), 5 % Sp (T_3) and 7 % Sp (T_4), three levels of *Nannochloropsis* genus (Nano) as follow: 3 % Nano (T_5), 5 % Nano (T_6) and 7 % Nano (T_7). Results indicated that T_6 had higher significant ($P < 0.05$) final body weight (FBW), average weight gain (AWG), specific growth rate (SGR) and relative growth rate (RGR) compared with the other experimental treatments. However, the best feed conversion ratio (FCR), was found in T_7. No mortality was observed in T_4 and T_7. The RBCs count was in favor of T_4 and T_3, and the leucocytes count T_5 and T_6, respectively compared to the control. The hemoglobin concentration related parameters (Hb, HCT and MCV) showed the highest significant values ($P<0.05$) in T_2, while the lowest values were recorded in T_4 and T_7. Farther, the microscopic differences of hepatocytes were discernible between control and the other treatments. The histological observation on livers of all treatments was evident that algae addition to the feed protected liver health.

Keywords: *Spirulina, Nannochloropsis, liver, blood parameters, AST, ALT.*

Introduction

Feed is the largest expenditure item in both semi-intensive and intensive fish cultures, as it stands for about 50% to 70 % of the total operational cost in a fish farm (**El-Sayed**, **2004**). For culturing fish in captivity, nothing is more important than sound nutrition and adequate feed. Nile tilapia, in particular, is naturally accustomed to eating plant ingredients (**Keenleyside**, **1991**). In a role to reduce feed costs **Webster *et al.* (1992)** replaced fish meal by plant protein sources in catfish diets. They combined two plant protein sources (SBM and distillers grains with solubles) and found no changes in growth performance and feed utilization parameters, and they concluded that SBM plant protein sources (soybean meal and distillers grains with soluble) can totally replace fish meal in a channel catfish diets. Recently, the use of feed additives has proven to be a successful application in aquaculture on growth performance and feed utilization without adverse effects on the environment as the using of antibiotics (**Meurer *et al.*, 2009)**. Several microalgae are source of proteins, carbohydrates, lipids, pigments, principally carotenoids (β-carotene, lycopene, cryptoxanthin), vitamins (A, B1, B2, B6, B12 and C), canthaxantin, astaxanthin, lutein and of phycobiliproteins (phycocyanin, phycoerythrins). As well as, source of minerals, polysaccharides, eight major amino acids, essential fatty acids specially γ-linolenic acid (GLA) required for complete nutrition. In addition to, secondary metabolites such as phenolic compounds, terpenoids, halogenated compounds, sulfur derivatives, and nitrogen derivatives; they have been used as feed additive not only in animal feeds and aquaculture but also in human nutrition as pharmaceutical ingredient in diet supplements and cosmetics (**Letsiou *et al.*, 2017; Sharifah and Eguchi, 2011)**. Thus, algae have immune system enhancing properties among other medicinal benefits **(Guillerme *et al.*, 2017 and Howe *et al.* 2006)**.

Spirulina has been used at a low-level as feed additive in order to improve the taste, texture and color of fish. Moreover, it is recommended for its positive effects on growth, feed utilization, physiological condition, stress and disease resistance (**Mustafa *et al.*, 1994)**. Hence, it has been used as a nutrient for fish larvae (**Lu** and **Takeuchi, 2004**) and as an ingredient in fish diets for juveniles and adults (**Palmegiano *et al.*, 2008)**. Some reports showed improved growth activity with the inclusion of *spirulina* in fish diets (**Manoher, 2005**).

Furthermore, it has been reported that *Spirulina* enriched diets have health benefits such as; antiviral, anti-inflammatory, anti-cancer in humans, animals and fish; as well as, preventing or managing hypercholesterolemia, hyperglycerolemia, allergies, diabetes, environmental toxicant, cardiovascular diseases and other metabolic disease among others (**Deng and Chow, 2010)**. *Nannochloroposis spp*. are produced on large scale for industrial usages as biodiesel, as well as pharmaceuticals and aquaculture for using EPA and carotenoids among other high nutritious material; which act as immune enhancer, cell growth, collagen production promotor, antiaging and bioactive ingredient for prophylaxis against various diseases (**Letsiou *et al.*, 2017).** Several researchers such as **Faggio *et al. (*2013) and (2014)** stated that, haematological parameters are important to assess the physiological status of fish and to monitor stress and pathological changes**.** Hence**,** blood plasma proteins are the main blood components which are synthesized in liver, spleen, plasmatic cells and lymphatic ganglions. Plasma proteins play pivotal role in regulating body functions such as, maintaining the normal volume and osmotic pressure of the blood, acid-basic equilibrium of blood, contribute in specific and nonspecific immune defense, work as enormous proteins reserve **(Patriche *et al.*, 2009)**. The total protein level is one of the indicators that reveal the nutritional status of organism. Furthermore, it gives qualitative and quantitative information regarding fish health which, correlated with age, sex, stage of sexual maturity and water temperature **(Patriche *et al.*, 2009).**

The aim of this work is to investigate the effect of substitution of soybeans with two species of algae (*Spirulina spp*. and *Nannochloropsis*) on the immunological and general health of *O. niloticus*.

Materials and methods

This work was conducted in Fish Production Branch, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, to evaluate the partially substitution of three levels (2, 5, 7%) of two algae species (*Spirulina spp*. (Sp) and *Nannochloropsis* genus (Nano) instead of soybeans and wheat brane, respectively. Growth performance, general health indicators through haematological analysis and histological parameters of tilapia (*Oreochromis niloticus*) were examined. Mono-sex *O. niloticus* fingerlings were obtained from World Fish Center, El-Abbassa, Sharkia Governorate, Egypt. Fish were distributed into 21 rectangular fiberglass tanks 60 × 30 × 60 cm represent 7 treatments with three replicas / treatment (negative control (T_1), three levels of *Spirulina spp*. (spiru) 3 % spiru (T_2), 5 % spiru (T_3) and 7 % spiru (T_4), three levels of *Nannochloropsis* genus (nanno) as follow: 3 % nanno (T_5), 5 % nanno (T_6) and 7 % nanno (T_7), respectively, with stocking density of thirty fish / treatment, with average initial body weight 2.7 g . Fish were fed a basal experimental diet for two weeks as adaptation period and then experimental diets were introduced to the fish for 93 days. Water parameters were maintained according to the following criteria: average water temperature 27 ± 2 °C, minimum dissolved oxygen 5 mg / L and pH 7.4. All fish in each treatment were weighed every two weeks during the whole experimental period. According to the data of body weight, the following parameters were estimated: average weight gain (AWG), feed conversion ratio (FCR) and specific growth rate (SGR) and were calculated according to (**Cho and Kaushik, 1985).**

Spirulina was obtained from Phytoplankton Laboratory, Limnology Department, Central Laboratory of Aquaculture Research; Agriculture Research Center, Egypt and Nanochloropsis were obtained from

Biotechnology Lab, National Research Council (NRC) Egypt. The chemical composition of the two algae species are shown in **Table 1**.

Table (1). Algae chemical composition on dry matter basis

	Spirulina	*Nannochloropsis*
Moisture (%)	10	10
Crude protein (%)	43	17.5
Crude fiber (%)	4	3
Ether extract (%)	3.5	18
Ash (%)	11	15
NFE (%)	28.5	36.5

Experimental diets

The formulations of the experimental diets are shown in **Table (2)**. The chemical analyses of the experimental diets were done to estimate dry matter (DM), crude protein (CP), ether extracts (EE), crude fiber (CF) and ash according to the method described by (**AOAC, 2007**).

Table (2). Formulation of the experimental diets

Ingredients	T1 (Negative control)	T2	T3	T4	T5	T6	T7
Fish meal	15	15	15	15	15	15	15
Soymeal 48	39	36	34	32	39	39	39
Wheat middling	25	25	25	25	22	20	18
Rice bran	7	7	7	7	7	7	7
Yellow corn	9	9	9	9	9	9	9
Fish oil	1	1	1	1	1	1	1
Plant oil	1	1	1	1	1	1	1
Spirulina	0	3	5	7	0	0	0
Nannochloropsis	0	0	0	0	3	5	7
Salt	1	1	1	1	1	1	1
Premix	1	1	1	1	1	1	1
limestone	1	1	1	1	1	1	1
Proximate analysis (%)							
Dry matter	91.3	91.1	91.3	912	91.3	91.1	91.3
Protein	34.00	33.8	33.7	33.6	34	34.1	34.1
Ether extract	5.29	5.37	5.42	5.47	5.34	5.37	5.4
Crud fiber	4.07	4.07	4.07	4.07	4.07	4.08	4.09
Ash	10.37	10.46	10.75	11.08	11.29	11.76	12.69
NFE	37.57	37.4	37.36	36.98	36.6	35.79	35.02

Growth parameter calculations

Average weight gain (AWG)

Daily weight gain was estimated according to the following formula:

$$AWG = (Wt_2 - wt_1)/ t$$

Where: wt_1 = first fish weight in grams.

wt_2 = following fish weight in grams.

t= period in day.

Specific growth rate (SGR)

Specific growth rate was calculated according to **Jauncey and Rose (1982).**

$$SGR = (Ln\ wt_2 - Ln\ wt_1)\ x100/t.$$

Relative growth rate (RGR)

Relative growth rate was calculated according to the following equation:

$$RGR = (wt.2-wt.1)\ x100/wt.1$$

Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) = feed intake/weight gain

Blood sampling

At the end of the experimental period, twelve O. *niloticus* juveniles from each experimental group were randomly taken. Blood were collected from each individual trough caudal venous puncture with the use of 1ml syringe previously washed with K_2EDTA; the sampling tubes contained K_2EDTA as anticoagulant (**Faggio *et al.*, 2014**).

Haematological and biochemical analysis

Blood samples of four fish were pooled to represent one tank and give enough amount for analysis. Samples were kept on 4 ºC until processed 6 hours after collection in three replicas by the same operator. Hematological parameters such as: haemoglobin (Hb), packed cell volume (HCT and PCV), red blood cell (RBC), white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH). mean corpuscular haemoglobin content (MCHC) was performed by using automated blood testing machine auto-haematology analyzer Rayto model RT7200. The rest of the samples were centrifuged at 3500 rpm for 20 min. to obtain blood for assaying the aspartate transaminase (AST), alanine transaminase (ALT), total protein (TP) and albumin (Alb), alkaline phosphatase (ALP), by (Biomed Diagnostic, Egypt) spectrophotometric commercial kits, according to the manufacturer's instructions. By vitroscient semi-automated clinical chemistry analyzer model VS10. Globulin was calculated according to the following "Globulin (total protein – albumin)"

Histological Investigation

Six fish from each were dissected to obtain liver, stomach and intestine, which were cut into small pieces and fixed in Davidson's modified solution (**Fournie *et al.*, 2000)** for three days. Later, samples were dehydrated through a series of ascending concentrations of ethanol, embedded and blocked-in paraffin wax. 5µ transverse sections were cut and stained with hematoxylin and eosin protocol. The examined tissue samples / treatment were 3 organs x 3 replications x 5 examined fields in each slide for abnormalities detection by a light microscope and were photographed by a fluorescence microscope Leica DM2500, Germany.

Statistical Analysis

The data were analyzed by using the GLM procedure with Two-way analysis of variance (ANOVA). Results were expressed as means ± SE. Significance was accepted at P <0.05; Statistical analysis was performed by using SAS® 9.3. Differences among means were tested for significance according to Duncan's multiple rang test (**Duncan**, **1955**). The following model was used to analyze the obtained data:

$Yij= \mu + Ti + L_j + (Ti * L_j) + eij_k$

Where:

Yij =observation.

µ = the overall mean.

Ti= the fixed effect of treatments.

L_j = the fixed effect of levels.

$Ti * L_j$ = interaction between treatments and levels.

Eij_k= random error.

Results and Discussion

Growth parameters

Data in Table (3) showed the growth performance of tilapia fingerlings fed different levels of both spirulina and nannochloropsis algae. By increasing spirulina algae levels in fish diets from 3 to 7%, growth performance of tilapia fish was reduced significantly ($P < 0.05$). In both algae treatments, the lowest significant ($P < 0.05$) average weight gain (19.13 g / fish) was recorded in T_4, while the highest value (24.42 g / fish) was found in T_6. The present growth results agree with those of **Bravo-Tello *et al.* (2018),** who found that supplemented Atlantic salmon diet up to 5% *Nannochloropsis* diet had positive effect on its growth performance. On other hand**,** those results were in disagreement with **Sarker *et al.* (2018)** who found that no significant reduction in weight gain of tilapia fish when they added 3 and 5% nannochloropsis in diets, whereas reduction in tilapia weight gain was observed with increasing nannochloropsis levels higher than that. The highest SGR and RGR values have been achieved by feeding tilapia fingerlings diets included 5% nanno, where the differences were significant (*P < 0.05*) among all treatments. The results obtained for the ADG and SGR, are supported by **(Patterson *et al.,* 2013** and **Tibaldi *et al.,* 2015),** who demonstrated that dietary supplementation with various microalgae, including a related nannochloropsis species up to 25% is acceptable based on growth performance, nutrient utilization, carcass yields, organ weights, sensory evaluation, digestive enzyme activities and intestinal histological parameters. So, the reason for the improvement in growth rates for T_6 (nanno 5) may be explained by **(Schneider and Roessler, 1994**. **Khozin- Goldberg *et al.,* 2011)**, who reported that, *nannochloropsis* accumulates significant amounts of membrane-bound eicosapentaenoic acid (EPA). **Apt and Behrens (1999)** showed that *nannochloropsis* species are widely used as a feed in aquaculture and have been proposed for the commercial production of EPA. The major fatty acids in *nannochloropsis* are 14:0, 16:0, 20:4ὠ6 and 20:5ὠ5.

Table 3 showed that the inclusion of different levels of nannochloropsis and spirulina in tilapia diets had no significant effects (P < 0.05) on FCR. The reason may be due to that the levels of nannochloropsis and spirulina used in the present study were little pet lower than that required to achieve effects. These results are agreed with (**Milad *et al*., 2016**), who found that dietary supplementation of *Spirulina platensis* up to 10% had no effects on growth performance, digestive enzyme activities, humoral, skin innate immune responses and disease resistance in the great sturgeon (*Huso huso*), while these results are in disagreement with **Dernekbasi *et al***. (**2010**), who reported that FCR increased with increasing dietary *Spirulina* meal level and ranged from 2.31-1.09 in dose dependent manner. Their results demonstrated that dietary supplementation with *S. platensis* (up to 10% level) could be useful for maintaining the overall health status of great sturgeon. **Sørensen *et al***. (**2017**) reported that feeds with 20% alga had negative effect on feed intake, FCR, lipid and energy retention and health of the fish. The defatted *Nannochloropsis oceania* could be used around 10%, without affecting the performance of Atlantic salmon negatively.

In the present study no mortality was observed in nanno7 and spiru 7 (**Table 3**). Tilapia survival rate did not affect significantly (P < 0.05) by different feeding treatments. These results agree with **Sudaporn *et al***. (**2010**), who reported that *Spirulina* could improve growth, reduction of mortality; overall elements of fish quality, firmness of flesh, and brightness of skin color as well as improving the cost performance ratio of the fish feed.

Blood Parameters

Blood analysis using automatic method is already used in the veterinary field; as well as blood cell counting and other parameters were performed by several researchers on wide variety of fish species such as mullet (*Mugil cephalus*) and gilthead sea bream (*Sparus aurata*) (**Faggio *et al.*, 2013 and 2014 and Fazio *et al.*, 2013**). Moreover, the first author showed that, there was no significant difference between manual and automated methods in this aspect. Blood parameters as revealed in (**Table 4 and 5**) showed that supplementing diets with spiru and nanno had significant effect on all the measured parameters ($P < 0.05$) among all treatments. The RBCs counts in spiru treatments were the highest in all doses with highest record in T_4 and T_3 (1.8 and 1.81*10^6 / mm^3), respectively. Meanwhile, T_7 and T_6 showed the highest recorded RBCs count for Nano treatment (1.74 and 1.58*10^6/mm^3), respectively, and the least count was obtained by the control (1.25*10^6/mm^3). The Hb and Hct values were the highest in treatments T_2 and T_3 followed by T_5 and T_6 compared to control. The hemoglobin concentration related parameters had significant effect in most treatment groups compared to the control except for T_2 and T_5 compared to other treatments in MCV, while control recorded the highest value in MCH and MCHC, respectively followed by other treatments. In addition, the total WBCs count in T_5 showed the highest value among all treatments (78.21*10^3 / mm^3) and the least was recorded by T_7 (62.70*10^6 / mm^3). It was interpreted from the scholars research that marine organisms are rich sources medicinal agent; thus, algae additive such as spirulina or nannochloropsis have therapeutic and restoration effect because of various biologically active metabolites **(Guillerme *et al.*, 2017 and Howe *et al.*, 2006)**. Further, they are highly nutritious due to, containing all the essential amino acids, easily digestible proteins and reduce anemia severity through hematopoiesis stimulation (**Berlean *et al.*, 2014**). The latter result is due to phycocyanin's elevated concentration, which mimics the effect of endogenous erythropoietin. The outcomes of **Mahmoud *et al.* (2018)**; proclaimed that lower doses range from 1 to 2% spirulina supplement raised both RBCs and WBCs count in *O. niloticus*.

Table (3). Growth parameters indices obtained by the experimental treatments.

	Control	Spirulina			Nannochloropsis		
	0	3	5	7	3	5	7
IBW	2.73± 0.09	2.56± 0.06	2.6 ± 0.11	2.73 ± 0.2	2.84 ± 0.09	2.8 ± 0.2	2.66 ± 0.1
FBW	25.63^{b} ± 1.5	24.7^{bc} ± 0.8	24.22^{bc} ± 1.5	21.86^{d} ±0.3	23.3^{c} ± 1.8	27.22^{a} ± 0.9	24.97^{bc} ± 0.6
AWG	22.9^{b}± 1.6	22.14^{bc} ±0.8	21.62^{c} ± 1.4	19.13^{e} ± 0.4	20.46^{d} ± 1.8	24.42^{a} ±0.8	22.31^{bc} ± 0.5
SGR	2.41^{a}± 0.06	2.44^{a}± 0.06	2.4^{a} ± 0.06	2.24^{b} ± 0.06	2.26^{b} ± 0.06	2.45^{a} ±0.06	2.41^{a} ± 0.06

FCR	1.47± 0.13	1.38± 0.13	1.52 ± 0.13	1.77 ± 0.13	1.79 ± 0.13	1.42 ± 0.13	1.37 ± 0.13
Survival	96.7 ±1	98.6 ± 1	97.9 ±1	100 ± 1	99.4 ± 1	98.6 ± 1	100 ± 1

Means in row with different superscript letters are significantly different (P < 0.05).

Table. (4). Haematological indices for experimental treatments Vs. control

Parameter	RBCs (*10^6 / mm^3)	Hb (g/dl)	HCT (%)	MCV (Fl)	MCH (pg)	MCHC (g/dl)	WBCs (*10^3 / mm^3)
T_1 (Control)	1.25^b	10.10^b	18.81^d	150.5^a	80.82^a	53.70^a	65.79^c
T_2	1.78^a	10.80^a	26.87^a	150.61^a	60.53^d	40.19^c	76.82^a
T_3	1.80^a	10.40^{ab}	26.05^{ab}	144.80^b	57.80^e	39.93^c	68.99^b
T_4	1.81^a	10.00^b	25.28^{ab}	139.90^d	55.32^{fe}	39.56^c	65.33^{cb}
T_5	1.34^b	10.50^{ab}	20.04^d	149.20^a	78.17^b	52.40^a	78.21^a
T_6	1.58^{ab}	10.50^{ab}	23.29^c	147.10^b	66.32^c	45.09^b	77.99^a
T_7	1.74^a	10.00^b	24.78^{bc}	142.10^c	57.34^{fe}	40.35^c	62.70^d
± **SE**	0.11	0.19	0.54	0.64	0.67	0.80	1.00

- Hemoglobin (Hb), hematocrit (HCT), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), red blood corpuscles (RBCs) and white blood corpuscles (WBCs).
- Means in column with different superscript letters are significantly different (P < 0.05).

Blood chemical analysis indicated that liver enzymes AST and ALT for the control treatment were 380±8.6 and 100±2.14, respectively. While the treated groups showed high decline on both of them especially in T_2 (80±8.6) and T_7 (110±8.6) for AST and (20±2.1) in T_3 and T_7 for ALT. Otherwise, control obtained the highest TP, while the lowest recorded by T_7., while T_5 had the highest recorded Alb value among all treatments. As for Glob value, the control had the highest value followed by T_6 and T_4, respectively. The findings of **Sharma *et al*. (2018)** suggested that, Antioxidant-rich supplements possibly decreased total protein in the serum. As well as the finding of **Enas *et al. (2020)* and Mahmoud *et al*. (2018)**, who stated that the experimental groups, which received treatment with supplementation of Spirulina or algae supplements had lower blood total protein compared to the control.

Table. (5). Experimental groups blood plasma chemistry indices Vs. control

Parameter	AST (U/L)	ALT (U/L)	TP (g/dl)	Alb (g/dl)	Glob (g/dl)
T_1 (Control)	380.00^a	100.00^a	12.00^a	2.70^b	9.30^a
T_2	80.00^f	40.00^c	4.90^e	1.90^d	3.00^f
T_3	140.00^d	20.00^d	6.30^d	2.70^b	3.60^e
T_4	200.00^c	100.00^a	6.60^{dc}	2.40^{cb}	4.20^d
T_5	320.00^b	97.00^a	10.30^b	3.50^a	6.80^b

T_6	312.00^{b}	60.00^{b}	7.60^{c}	2.70^{b}	4.90^{c}
T_7	110.00^{e}	20.00^{d}	4.60^{e}	2.00^{cb}	2.60^{g}
± **SE**	8.6	2.1	0.36	0.15	0.1

- Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), plasma albumen (Alb) and plasma globulin (Glob).
- Means in column with different superscript letters are significantly different ($P < 0.05$).

Histological examination

The examination of intestinal and stomach tissue sections revealed no striking macroscopic differences between control and other treatments (**Fig. 1 and 2**). In addition, the liver tissue transverse sections examination showed, hepatocytes, hepatopancreas and central vein (**Fig.3, a**).

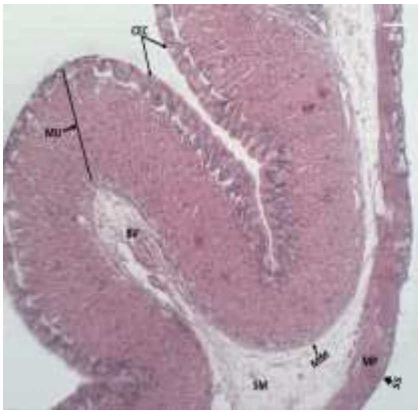
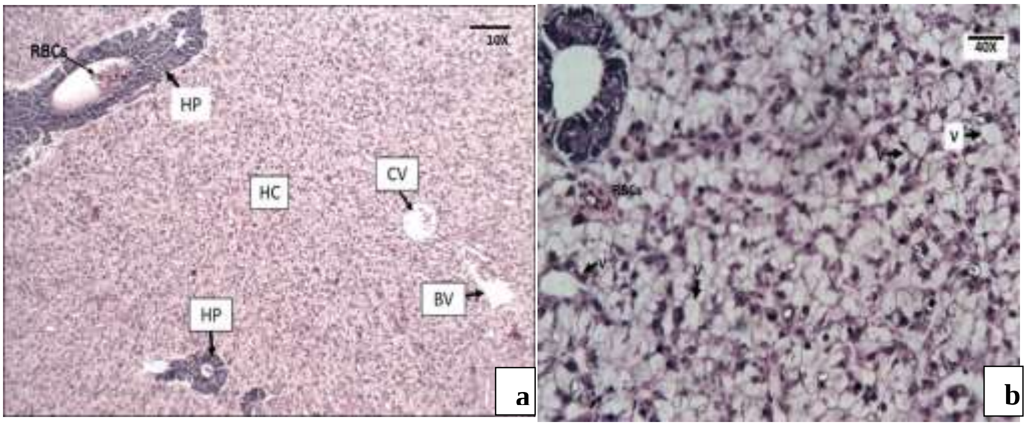


Fig. (1). Cross section in Nile tilapia stomach stained with H&E; showing stomach normal architecture in both control and treated groups; Blood vessel (BV), columnar epithelial Cells (CEC), mucosa (Mu), muscularis mucosa (MM), muscularis propria (MP), submucosa (SM), serosa (SS).



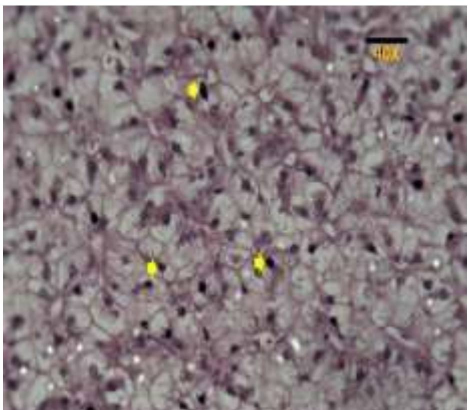


Fig. (3). a) Liver cross section H&E stained showing treated groups intact membrane structure and clear centric nuclei without significant abnormalities; Blood vessel (BV), Central vein (CV), Hepatic cells (HC), HP= Hepatopancreas, Red blood corpuscle (RBCs).
b) Cross section of control group liver H&E stained showing mild lipid deposition vacuoles (V) and peripheral displacement of the nuclei.
c) Cross section of treatments liver H&E stained yellow arrows refer to Kupffer cells.

The microscopic differences were discernible between control and the other treatments, where the control hepatocytes exhibit mild lipid deposition vacuoles and peripheral displacement of the nuclei (**Fig.3, b**); in addition to slight degenerative areas showed in some individuals. Whereas the livers from tilapia fed on spirulina or nannochloropsis with concentration 3, 5 and 7% respectively; showed intact membrane structure and clear centric nuclei without significant abnormalities as well as, smaller cell volume than the control group in the same microscopic field area of vision (**Fig.3, a and c**). The histological observation on livers of all treatments was evident that algae addition to the feed had protect liver health, prevents the development of the fatty liver through its hypocholesterolemic effect as shown by the findings of **El-Feky (2017); Sangeetha *et al*. (2017)**. AST and ALT enzymes normally exist within liver cells. If the liver is injured or damaged, the liver cells release these enzymes into the blood, raising the blood AST and ALT levels as a signal for liver disease (**Sangeetha *et al*., 2017 and Bhattacharyya and Mehta, 2012)**. It was found in this study that the usage of spiru and nanno algae in all treatments reduced the liver AST and ALT dramatically, which also support the histological findings. That come in agreement with the findings of several research demonstrated the hepatoprotective effect of algae additives such as **El-Sheekh *et al*. (2014); Bhattacharyya and Mehta (2012) and Ferreira-Hermosillo *et al*. (2010).**

Conclusion

The results of this study on cultured tilapia showed that using amount of spirulina or nannochloropsis for 95 days did show positive significant effect on some growth parameters, hematological and immuno-physiological indices between treatments. Moreover, the usage of dried algae or algae extracts to enhance fish hematological and immuno-physiological indices requires more comprehensive research about the dose and synergism with other feed additives.

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الملخص العربى

تعزيز المؤشرات المناعية والصحية العامة لأسماك البلطي النيلي من خلال الإحلال الجزئي لفول الصويا بطحلبي الاسبيرولينا والنانوكلوربسس

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كان الهدف من هذه الدراسة هو تقييم تأثير الإحلال الجزئي لكل من طحلبي الاسبيرولينا و النانوكلوربسس بدلاً من كسب فول الصويا على أداء النمو و مؤشرات الصحة العامة لأسماك البلطي من خلال التحليل الدموي والدراسة النسيجية من خلال سبعة معاملات على النحو التالي: المجموعة المقارنة (المعاملة الأولى) ، ثلاثة مستويات من طحلب الاسبيرولينا: 3 % اسبيرولينا (المعاملة الثانية)، 5 % اسبيرولينا (المعاملة الثالثة) و 7 % اسبيرولينا (المعاملة الرابعة) و ثلاثة مستويات من جنس النانوكلوربسس على النحو التالي: 3 % نانو (المعاملة الخامسة) ، 5 % نانو (المعاملة السادسة) و 7 % نانو (المعاملة السابعة). أشارت النتائج إلى أن المعاملة السادسة كانت أعلى معنويا في وزن الجسم النهائي، متوسط زيادة الوزن ، معدل النمو النوعي و معدل النمو النسبي مقارنة بالمعاملات التجريبية الأخرى. ومع ذلك ، تم العثور على أعلى معدل تحويل (الأفضل) في المعاملة السابعة. وجد أنه لا يوجد وفيات في المعاملة الرابعة والمعاملة السابعة. أظهرت القياسات المتعلقة بتركيز الهيموجلوبين و (HCT) و (Hb) و (MCV) أعلى القيم المعنوية بين معاملات الطحالب في المعاملة الثانية ، بينما سجلت أدنى القيم في المعاملة الرابعة والسابعة. بالإضافة إلى ذلك ، كان عدد كريات الدم الحمراء لصالح كل من المعاملة الرابعة والثالثة ، وكرات الدم البيضاء في المعاملة الخامسة والسادسة ، على التوالي مقابل المجموعة الضابطة. إلى جانب ذلك ، يمكن تمييز الاختلافات المجهرية لخلايا الكبد بين المجموعة الضابطة والمعاملات الأخرى . كانت الملاحظة النسيجية على كبد جميع المعاملات واضحة أن إضافة الطحالب إلى العلف قد حمت صحة الكبد.