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Nutritional Effects of Dietary Spirulina (*Arthrospora platensis*) on Morphological Performance, Hematological Profile, Biochemical Parameters of Common Carp (*Cyprinus carpio* L.)

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THIS research was done to find the effect of spirulina (*Arthospira platensis*) on some physicochemical parameters in common carp (*Cyprinus carpio*). Fish with an initial body weight of 510.3±7.02g were collected from a private farm in Khank Township, Sumeel District, Duhok Government, Iraq. Fish were separated into two groups (twenty fish in each group), the carp in the first group fed on pellets alone while the second fish group fed on pellets with 10 mg/kg spirulina for 90 days. Results; there was no significant change of morphological parameters in fish feeding with commercial spirulina pellets compared with control fish group. In contrast, a significantly increases in the average daily and total gain, the specific growth rate and the condition factor in the fish fed commercial pellets with spirulina compared with control fish. Regarding the complete blood pictures, a significantly higher of the red and white blood cells, hemoglobin, and hematocrit observed in spirulina fish compared with the first group while a significant decreases of the mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration in fish feds commercial pellets with spirulina. Furthermore, highly significant differences were seen among lipid profile parameters with exception of significantly reduce of low-density lipoprotein in fish fed diets with spirulina. Moreover, significant increases levels of the total protein and globulin were observed in spirulina fish compared with the first group while a reduction of albumin level was noted. In addition, a significant reduction of the level serum enzyme activities of fish fed with commercial pellets with spirulina compared with the control fish. This study concludes that the diet with spirulina supplementation has a crucial roles in growing performances and hematological and biochemical parameters in fish.

Keywords: *Spirulina, Arthospira platensis, Cyprinus carpio, Morphological parameters, Enzyme activities.*

Introduction

In recent years, one of the fields providing animal protein that has been developed through aquaculture. Globally, including the Middle East and Iraq, one of the most widely domesticated aquatic fishes is the common carp (*Cyprinus carpio* L.). Furthermore, common carp meat has a distinct flavour and is simple to digest. This fish is certainly omnivorous, resilient, and adaptable to a variety of different environmental conditions [1]. Previous studies reported that Common carp has two different subspecies, C. *c. carpio* from Europe ⁄ Central Asia and C. *c. haematopterus* from East Asia, as demonstrated by recent molecular genetic research [2,3]. Various organic substances produced by vegetation provide the most effective and safer options for healthcare [4]. According to a recent study by Sirakov [5], confirmed that algae are one of the easily obtainable and cheap foods that might be solved the problem created by the fish farming industry. Free-floating filamentous cyanobacteria recognized as spirulina, have been discovered in Africa, Asia, South and Cen-

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tral America, and Europe in lakes of tropics and subtropics. They are categorized by their cylindrical, eukaryotic trichomes in an open left-hand helix [6]. Studies have demonstrated that Spirulina has a large number of antioxidants, including beta-carotene, phycocyanin, tocopherols, and micronutrients and also contain (55-70%) protein and (5-6%) lipid [7,8]. Polyunsaturated fatty acids (PUFAs) consist of 1.5-2% of the lipid form [9]. Spirulina has been shown to be effective in preventing various diseases such as microbial infections, cancer, hypersensitivity, and diabetes and due to its highly dietary intake, this microalga was preferred by both the European Space Agency (ESA) and National Aeronautics and Space Administration (NASA) is an important diet for long term space missions[10].

Oxidative stress happens when a cellular system fails to eliminate oxygen free radicals accumulated from metabolism [10]. These radicals damage proteins, lipids, and nucleic acids, by attacking them, affecting the disruption of energy metabolism, cell transport, and the signalling process [11]. This oxygen-free radical accumulates and causes necrosis via the programmed cell death pathway [10].

The current study showed that assessment of the nutritive quality and growth activity of the fish. In addition, this study also highlights to determine the effect of Spirulina on some basic haematological, biochemical, and enzyme activities.

Material and Methods

Study areas

The greatest dam in Iraq is Mosul Dam. It is situated in the western Ninawa. The selected place was chosen in Khank Township, Sumeel District, Duhok governorate, Kurdistan Region, because it is considered the greatest portion of rearing fish using cage fish culture, and numerous circular cage fish farms were constructed there [12, 13].

Experimental design and diet composition

Forty common carp (*Cyprinus carpio*) from both sexes with an initial body weight of 510.3±7.02 g were collected from a private farm in Khank Township, Sumeel District. In the aquaria room, the fish were transported and placed in indoor plastic tanks (1x1x1m), each holding 100L. Before starting the experimental trial, the fish undertook a 30-day acclimation period in a regular laboratory environment. The tap water was used to fill the plastic tanks (water temperature 23.4^{o}C, pH 7.9, O_2 89.24 %, and salt content 0.07 g\L) with continuous aeration added to each tank utilizing little air pumps for aquarium. The foods consist of typical items presented in Summel District shops, having 32% total protein. *Spirulina platensis* powder of the highest quality was obtained from (Natura Vitalis, Netherlands). According to Abdel-Tawwab and Ahmad [14], Each food's component acquired 100 cc of water with microalgae mixed in it. It was combined to create a paste of each food. The pastes were split as parts, allowed to dry for a few days at room temperature, and then ground to create little pellets. The pellets were kept in plastic containers and air-dried. The dietary experiments started on July 8th, 2022; the fish were fed daily in the morning in a ratio of (10 mg/kg body weight/day) over 90 days. The uneaten food and fecal contents were removed, and then no death was documented during the trial. Later the period of acclimation, the fish were separated into two groups, both containing twenty fish. The carp in the 1st group nourished on the standard feed pellets, whereas the carp in the second group nourished on the standard feed pellets with 10 mg\kg spirulina.

Morphological Parameters

The fish's length was calculated by setting it on the ruler with its snout at the zero-centimetre mark. Fish can be measured for three different lengths: Total length (TL) (calculated from the snout to the caudal fin) and standard length (SL) (calculated from the snout to the end of the tail), and fork length (FL) (calculated from the snout to the middle of a concave tail) [15].

Furthermore, the average total gain (ATG), specific growth rate (SGR), condition Factor (K), and average daily gain (ADG) were measured as stated by these equations.

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

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

K = (100*w)/ L3

ADG (g/fish/day) = {ATG (g)/experimental period (d) [16].

Hematological and Biochemical Parameter

After the exposure period, blood tests (3ml) were directly collected from a fish's caudal vein

utilizing both heparinized and non-heparinized syringes (for haematological and biochemical investigation, respectively). Then blood poured into heparinized plastic tubes to determine blood components by using automated haematology analyser (Sysmex XP-300™ Coulter, USA) in accordance with the instructions provided by the manufacturer. For biochemical and enzyme activity measurements, the blood was immediately centrifuged at 1500 rpm for 5 minutes to get serum for analysis. The enzyme activities and biochemical parameters were assayed with the aid of FUJIFILM (DRI_CHEM NX500-Cezch Republic) following the manufacturer's instruction of the slide reagent kits.

All statistical analyses were performed with the GraphPad Prism software (Version 8). The morphological performances were performed by Dunnet-tests of One-way analysis of variance (ANOVA).

The effects of spirulina on haematology and biochemical parameters were assessed by paired t-test was used to compare the two groups, and the data were presented as means and standard error of the mean (SEM).

Results

As indicated in Fig. (1) revealed that although the fish fed on a diet with 10 mg/kg spirulina had a slight increment in the body weight, body length, and condition factor, there were not statistically significant observed when comparing with carp fed on the normal diet. However, later 90 days of fish feeding with spirulina, there were markedly increases ($p < 0.05$) in average of total gain, daily gain, and feed convention ratio (Fig. 2).

Haematological Profile

Figure (3) reveals the common carp haematological profile with several parameters, this demonstrates that feeding fish a commercial spirulina diet for 90 days causes a significant increase in the fish's RBCs and WBCs numbers as well as their haemoglobin and haematocrit when compared with fish feds on commercial pellets alone. In addition, the concentration of MCV, MCH, and MCHC demonstrated a statistically significant ($p<0.01$) reduction in fish-fed diets with 10 mg/kg spirulina when comparing with the fish-feeding commercial diets. In the other hand, there was no significant change in the percentage of the lymphocyte after feeding the fish with a commercial diet plus spirulina with the control group.

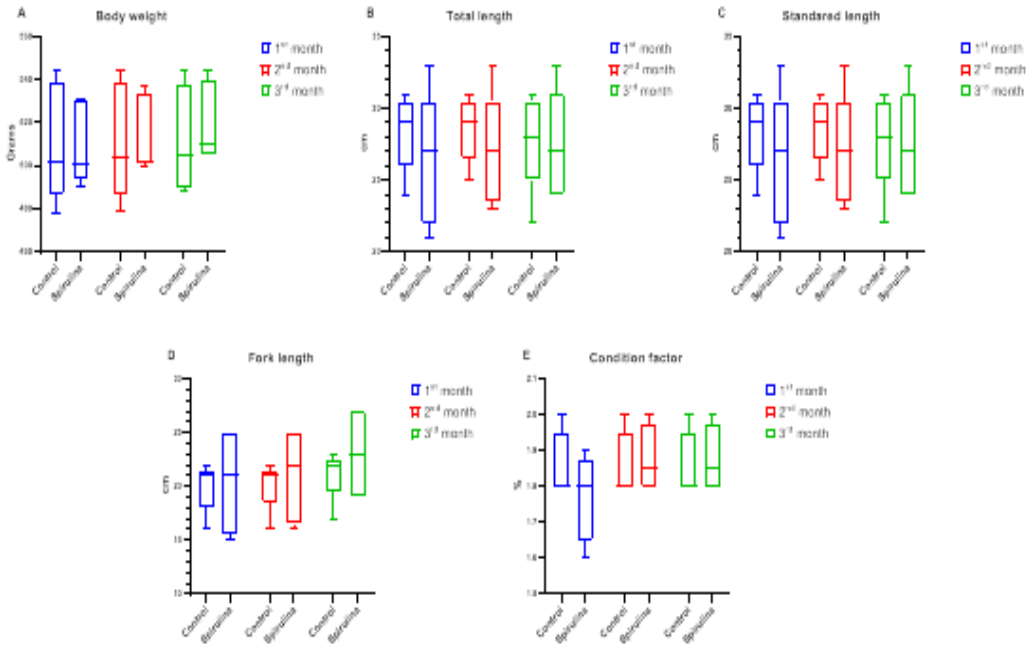

Fig. 1. Comparison graph for morphological parameters; body weight (A), total length (B), standard length (C), fork length (D), and condition factor (E), in fish feds commercial pellets with 10mg/kg of spirulina compared with fish feds commercial pellets alone.

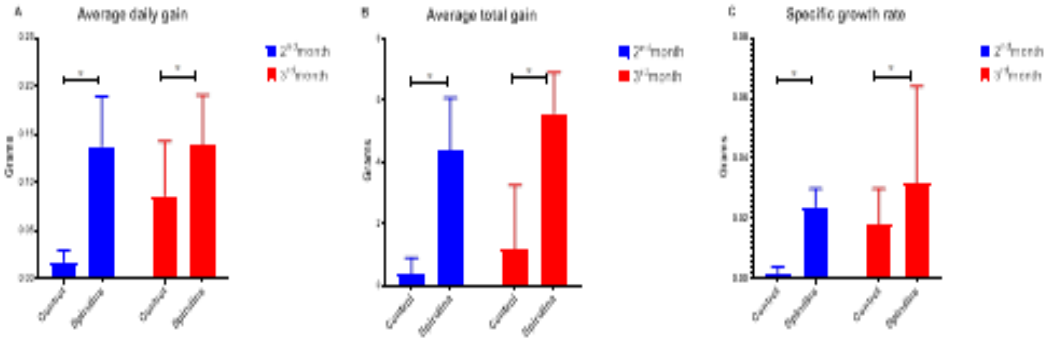

Fig. 2. Comparison graph for average daily gain (A), average daily gain (B), and specific growth rate (C), in fish feds commercial pellets with 10mg/kg of spirulina compared with fish feds commercial pellets alone. *p<0.05.

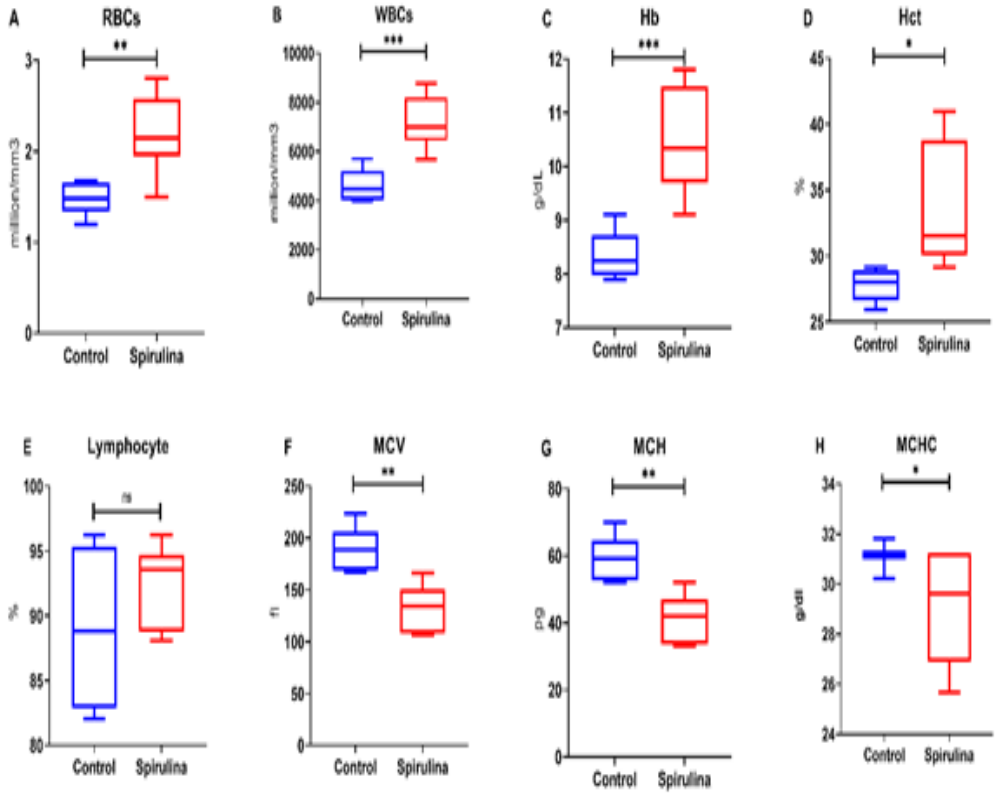

Fig. 3. Comparison graph for haematological profile; Red blood cells (A), white blood cells (B), haemoglobin (C), haematocrit (D), lymphocyte (E), mean corpuscle volume (F), mean corpuscle haemoglobin (G), and mean corpuscle haemoglobin concentration (H) in fish feds commercial pellets with 10mg/kg of spirulina compared with fish feds commercial pellets alone.
Keys: *p<0.05, **: p<0.01, *** p<0.001 and ns; none-significant

Biochemical and Enzyme activities parameters

The data regarded of protein profile, which include the total protein (3.27±0.15), albumin (0.52±0.02), and globulin (2.74±0.16). Statistically, compared to the fish in the control group, the fish fed the commercial diet plus spirulina had considerably higher levels of total protein and globulin while albumin was reduced in spirulina fish group in contrast to the control group (Fig. 4)

Figure (5) shows the result for cholesterol, triglyceride and very low-density lipoprotein (VLDL) were significantly decrement ($P<0.001$) on fish-fed diet with spirulina compared with the fish feds normal pellets while the levels of high-density lipoprotein (HDL) was statistically ($p<0.01$) elevated on the fish feds diets with spirulina (98.4±3.71) when compared with the control group (41.7±2.3). concerning the level of Low-density lipoprotein was observed not changes in the fish fed 10mg/kg spirulina as comparing with the fish fed commercial pellets alone.

In addition, several enzymes' activities, (ALT, AST, ALP, and LDH), were also measured between the two groups (Fig. 6). It is interesting to note that the levels of these enzymes were markedly reduced in the fish fed on commercial diet plus spirulina as comparing with the fish fed commercial pellets alone.

Discussion

Recently studies have focused on microalgae as a potential replacement for fishmeal. Spirulina is rich in biochemical components such as essential fatty and amino acids, carotenoid, vitamins, and minerals for aquatic animals [17, 18]. Indeed, spirulina offers advantageous physiological impacts animals by providing an immune response and good growth performance weight [18].

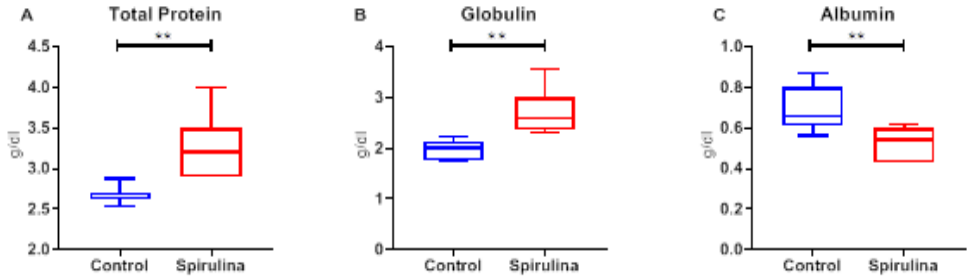

Fig. 4. Comparison graph for the total protein (A), globulin (C), and albumin (B), in fish feds commercial pellets with spirulina compared with fish feds commercial pellets alone. **: p<0.01.

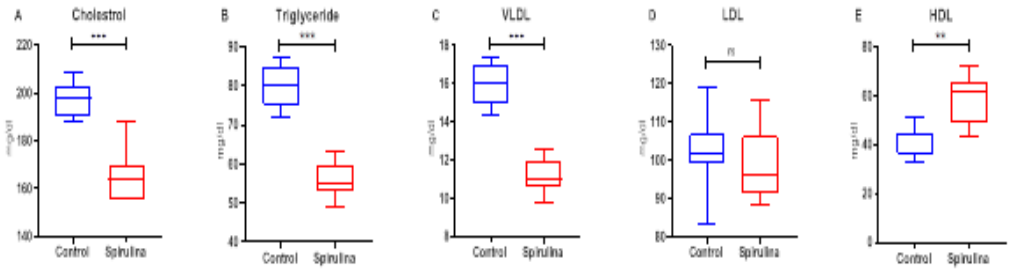

Fig. 5. Comparison graph for lipid profile; Cholesterol (A), triglyceride (B), very low density lipoprotein (C), low density lipoprotein (D), and high density lipoprotein (E) in fish feds commercial pellets with 10mg/kg of spirulina compared with fish feds commercial pellets alone.
ns; none-significant, *p<0.05, **: p<0.01, and *** p<0.001.

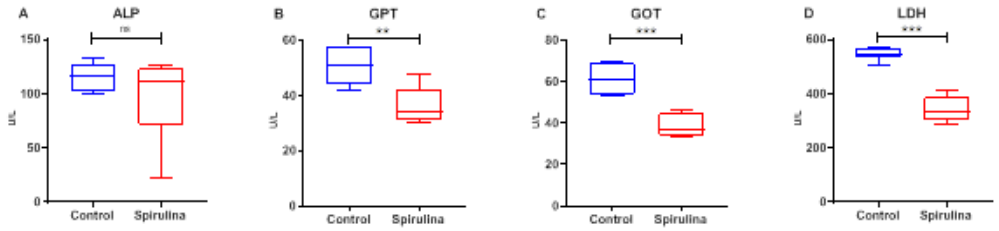

Fig. 6. Comparison graph for enzyme activities; alkaline phosphatase (A), glutamate pyruvate transaminase (B), glutamate oxaloacetate transaminase (C), and lactate dehydrgenaseDH (D) in fish feds commercial pellets with 10mg/kg of spirulina compared with fish feds commercial pellets alone.
ns; none-significant, **: p<0.01, and *** p<0.001.

Based on the findings of the current observations, the carp fed on control feed supplemented with 10 mg/kg spirulina produced a positive impact on body weight, total length, condition factor, ADG, ATG and SGR. These results agree with several conducted studies on the Nile tilapia (*Oreochromis niloticus*), in which authors discovered that the addition of spirulina in fish foods enhanced markedly the growth performance [19-23] as well as agree with results obtained by Dernekbasi [24], who examined the addition of spirulina in the guppy diet, and they found that raising the content of spirulina in the diet supplied superior growth compared to the other feed normal pellets. The positive impact in the value of RBCs, WBC, Hb, and Hct scored by the fish feeds on a diet with 10mg/kg body weight spirulina and these findings similar to study by Promya [25]. Promya and his colleagues reported that feeding *Clarias gariepinus* fingerlings with normal diet with 5% spirulina resulted in significantly rise of both RBCs and WBCs and immunity-stimulating capability values. It has been previously confirmed that the elevation in these parameters is due to C-phycocyanin's presence, which can improve immunity [26].

The total protein, albumin, and globulin ratio also significantly changed when fish feds diets with spirulina for 90 days. These incomes were analogous to the findings [27] who observed a considerable elevation in the ratio of TP for fish fed on standard pellets comparable to fish that are fed a diet with 5g of spirulina/kg that contains. Sherif et al.[28] indicated a high level of TP and globulin in groups containing spirulina and a decrease in albumin concentration in diets containing spirulina. Furthermore, two studies were discovered that fish-fed diets containing 10–5g of spirulina/kg had higher protein and globulin concentrations than fish fed the control diet [29, 30].

In this current study, the levels of lipid profile revealed a significant decrement in the rate of cholesterol, triglyceride, LDL, and VLDL and a significant elevation in the rate of HDL after feeding the fish's diet with spirulina. According to the findings, spirulina can be a great supplement to fish diets, and this is due to the highest blood ratio which involved in the immune response in fish [27]. Furthermore, spirulina could decrease the level of blood cholesterol, not the glucose, of common carp, and this was similar to the recent finding by Rehman [31]. They stated that spirulina involved in the metabolism of lipids, which cause to reduce the cholesterol. In addition, decrease in the enzyme activities (aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH)) in the blood of fish feds diets with spirulina could indicate a metabolic system that produces energy [32]. These findings of this investigation were in agreement with [33] that indicated a decrease in the level of ALT and AST enzymes. As previously recorded that biochemical markers of the liver (ALT and AST) in *Oreochromis niloticus* are influenced by this microalgae [34]. It is not doubt that the roles of spirulina acting as an antioxidant which prevent the formation of ion radicals [35].

The releasing of lactate dehydrogenase (LDH) and Alkaline Phosphatase (ALP) were decreased in the blood of fish feds diets with spirulina because the extract of these microalgae (phycocyanin) acts as a scavenger for removing the free radicals [36]. Besides, the presence of β-carotene may be responsible for Spirulina's beneficial role.

Very recent study by AlFadhly [37] reported that β-carotene in spirulina might decrease the cellular damage, practically deoxyribonucleic acid (DNA), thus playing a vital role in the regeneration of liver cell damage.

Conclusion

Using of spirulina (Arthrospora platensis) has an impact role in common carp health, with acceptable growth performance and increasing immune response capacity.

Ethics approval

This study was ethically approved by Animal Ethics Committee of Faculty of Science, University of Zakho (Code; AEC, 20/05/2022).

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Conflict of interest

There are no competing interests.

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The author didn't receive any fund

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التأثير الغذائي للسبيرولينا على الأداء المورفولوجي والخصائص الخلوية والكيميائية والانشطة الإنزيمة للدم في أسماك الكارب (*Cyprinus carpio* L.)

باسم سالم احمد
فاكولتي العلوم - جامعة زاخو- دهوك - إقليم كردستان - العراق

أجريت هذه الدراسة لمعرفة تأثير السبيرولينا (*Arthospira platensis*) على بعض العوامل المورفولوجية والدموية والكيميائية لسماك الكارب.

تم جمع الأسماك التي يبلغ وزنها البدائي ٥١٠,٣ ± ٧,٠٢ غم من مزرعة خاصة في قرية خانكي التابعة لقضاء سميل في محافظة دهوك ، العراق. قسمت الأسماك إلى مجموعتين (كل مجموعة تحتوي على ٢٠ سمكة) ، غذيت الأسماك في المجموعة الأولى على علف تجاري، بينما غذيت الأسماك في المجموعة الثانية على علف تجاري بعد اضافة ١٠ ملغم / كغم من السبيرولينا لمدة ٩٠ يومًا. أظهرت النتائج بأن الاسماك التي غذيت بعلف المخلوط بطحلب السبيرولينا زيادة طفيفة ($P> 0.05$) في المتغيرات المورفولوجية. من ناحية اخرى,فأن معدل المتوسط الكسب اليومي و معدل المتوسط الكسب الكلي و المعدل النمو النوعي وعامل الحالة في الأسماك لتي تتغذى على العلف المخلوط بسبيرولينا أظهرت زيادات معنوية ($p <0.05$). كما أظهرت البيانات بأن وجود السبيرولينا في النظام الغذائي ساهم بزيادة كريات الدم الحمر و البيض والهيموجلوبين والهيماتوكريت .هناك انخفاض معنوي ($p <0.05$) في كل من متوسط الهيموجلوبين العضلي ، ومتوسط حجم الجسم العضلي ومتوسط تركيز الهيموجلوبين في الأسماك التي تتغذى على السبيرولينا. كما لوحظت اختلافات معنوية في كل من البروتينات الكلية ، الألبومين ، الجلوبيولين ، وخصائص الدهون في علائق الأسماك التي تتغذى على السبيرولينا بالمقارنة مع الأسماك في المجموعة الضابطة. وكذلك حدث انخفاض كبير ($p <0.05$) أيضًا في إنزيمات المصل والفوسفاتيز القلوي بعد إضافة السبيرولينا في غذاء الأسماك. تبين بأن السبيرولينا يلعب دورا فعالا في النظام الغذائي للأسماك.