Effect of microalgae *Spirulina* spp. as food additive on some biological and blood parameters of common carp *Cyprinus carpio* L.

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Abstract

This study was carried out to study the effect of adding different levels of the microalgae *Spirulina* spp. in fish laboratory of Animal Sciences Department, College of Agricultural Sciences of Sulaimani University, Sulaimani, Iraq. A total of 72 common carp fingerling weight 45±2 gm used to detect the effect of three different levels of the algae *Spirulina* spp. The control treatment T1 with 0%., T2 with 3 gm /kg diet and T3 with 5 gm *Spirulina* /kg diet. Each treatment in three replicates in which six fingerlings common carp were stocked in each aquarium. Results indicated no significant differences at P<0.05 in RBC count, gonadosomatic index and hepatosomatic index. Diet T3 (5 gm/kg *Spirulina*) in T3 was higher significantly at P<0.05 in Hb, while control without any *Spirulina* was higher significantly at P<0.05 in each of platelets, MCV, MCH and MCHC. Adding of *Spirulina* to common carp diets in different levels leads to significant differences in differential WBC counts, T3 with 5 gm *Spirulina* /kg diet was higher in WBC counts. Lymphocytes were higher in each of control and T3. Monocytes were higher in control, granulocytes in T2 with 3 gm *Spirulina* /kg diet. Adding of *Spirulina* affected significantly in cholesterol as compared to the control. Triglyceride was higher in control and T2. T3 with 5 gm *Spirulina* /kg diet leads to higher and lower significantly in HDL and LDL respectively. T1 and T3 were higher significantly in spleenosomatic index. Inclusion of *S. platensis* in fish diet as a feed additive or as a partial replacer of the expensive fishmeal imposes significant promotions in fish growth, coloration, reproduction and flesh quality.

Keywords: *Spirulina*, Biological parameters, Blood parameters, common carp
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تأثير الطحلب الدقيق *Spirulina* كإضافة غذائية في بعض الصفات البايلوجية والدمية لأسماك الكارب الاعتيادي *Cyprinus carpio* L.

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الخلاصة

أجريت الدراسة الحالية لبيان تأثير إضافة مستويات مختلفة من الطحلب الدقيق سبيرولينا في مختبر الأسماك لقسم علم الحيوان، جامعة السليمانية، السليمانية/ العراق. تم إستخدام ٧٢ إصبعية لأسماك الكارب الاعتيادي بوزن ٤٥±٢ غم لدراسة تأثير ثلاث مستويات مختلفة من الطحلب الدقيق سبيرولينا. المجموعة الأولى اعتبرت مجموعة سيطرة ولم يتم إضافة الطحلب الدقيق سبيرولينا لعلف هذه المجموعة، المجموعة الثانية أضيف ٣ غرام من الطحلب الدقيق سبيرولينا / كغم علف، والمجوعة الثالثة أضيف ٥ غرام من الطحلب الدقيق سبيرولينا/ كغم علف. كل معاملة بثلاث مكررات وفي كل مكرر ست إصبعيات من الكارب الاعتيادي. حددت النتائج عدم وجود فروقات معنوية (P<0.05) في تعداد كريات الدم الحمراء، ودليل المناسل والكبد. كانت نتائج المجموعة الثالثة اعلى معنوياً في

الهيموغلوبين، بينما كانت معاملة السيطرة اعلى معنوياً في كل من معدل الصفيحات الدموية وحجم كريات الدم الحمراء (MCV) وكمية الهيموكلوبين في الكرية (MCH) وتركيز الهيموكلوبين الوسطي في الكرية (MCHC). أدت إضافة السبيرولينا إلى علف اسماك الكارب الاعتيادي بتراكيز مختلفة إلى وجود فروقات مختلفة في العد التفريقي لكريات الدم البيضاء، أما المجموعة الثالثة فقد كانت الأعلى معنوياً في تعداد كريات الدم البيض. كانت الخلايا اللمفاوية أعلى معنوياً في كل من السيطرة والمجموعة الثالثة، كانت الخلايا الوحيدة أعلى معنوياً في السيطرة، والخلايا الحبيبية في المجموعة الثانية. أثر إضافة الطحلب الدقيق سبيرولينا إلى زيادة معنوية تركيز الكوليسترول بالمقارنة مع السيطرة. كان تركيز الكليسيريدات الثلاثية أعلى معنوياً في السيطرة والمجموعة الثانية. أدت معاملة السيطرة والمجموعة الثالثة إلى ارتفاع وانخفاض معنوي في كل من البروتينات الدهنية مرتفعة الكثافة (HDL) والبروتينات الدهنية واطئة الكثافة (LDL) على التوالي. كانت مجموعة السيطرة والمجموعة الثالثة أعلى معنوياً في دليل الطحال.

Introduction

Fisheries have always played a very significant socioeconomic role in many countries and communities, as a subsistence product, fish are a vital resource towards poverty reduction and food security for poorer households (1). *Spirulina* as "the best for tomorrow", it is gaining popularity in recent years as a food supplement (2). The economic importance of cyanobacteria primarily lies in their agronomic importance as bio fertilizers due to N_2-fixation that helps them to grow successfully in habitats where little or no combined N is available, in recent times, their ability to produce structurally novel and biologically active natural products has been recognized (3-5). Certain cyanobacteria, known as extremophyles, inhabit extreme environments, e.g. *Spirulina* (alkalophilic), because of their special requirements, mass cultures of extremophyles are likely to be free from microbial contamination thus, avoiding a serious problem in outdoor cultures (6).

Earthrise farms, a commercial producer of *Spirulina* in California, estimate that Japanese fish farmers used about $2.5 million worth of *Spirulina* in 1989 (7). Their promotional literature lists the key benefits associated with the use of *Spirulina* in aquaculture (a) better growth rates are obtained, and less feed is wasted because of the inherent palatability of *Spirulina,* fish fed with this cyanobacterium have less abdominal fat, the energy being redirected into growth, this hypothesis was tested and verified in feeding trials with cherry salmon (7), and (b) fish fed *Spirulina* have an improved quality in terms of flesh flavor, consistency and color. (7) reports that sea bream *Acanthopagrus australis,* Mackeral *Acanthocybium solandri*, Yellowtail *Seriola lalandi,* and ornamental koi carp *Cyprinus carpio* exhibited enhanced coloration upon feeding with *Spirulina* supplements. A number of authors (8-10) have investigated the utilization of microalgae in the diet of aquaculture species. Phytobiotic are beneficial for modulation the growth performance and non-specific immunity of fish, based on the study of (11) the phytobiotic mixture composed of garlic and *Spirulina* is recommended as a potential nutraceutical and immunostimulant feed additives in cultured tilapia.

The aim of present study was to examine the effects of adding *Spirulina* to diet on some biological and blood parameters of *Cyprinus carpio*.

Material and methods

Experimental fish

The experiment was conducted for 42 days and for this purpose 72 fingerlings common carp *C. carpio* L. (weights ranged between 40-49 gm) were brought Daquq/ Kirkuk/ Iraq. The fish were sorted depending on size then weighed and put in experimental plastic tank. The fish were acclimated to laboratory conditions and fed with control pellets (31% protein) prior to the feeding trials for 21 days.

Experimental system and design

Twelve plastic tanks (100 L) were used in this trial. Each tank was provided with a proper continuous aeration. Each tank was stocked with six fish. The numbers of treatments in the trial were three with three replicates for each. The tanks (replicates) were randomly allocated to minimize differences among treatments. The continuous water flow discharged non-consumed feed and feces particles from the aquaria. In addition, a daily cleaning by siphon method was applied to remove remaining particles from the system.

Diet formulation

Experimental diets were prepared with the ingredients shown in table 1. The ingredients were mixed with water to obtain dough. Then, the dough was passed through an electrical mincer for pelleting by using Kenwood Multiprocessors. The pellets were dried at room temperature for a few days and crushed to yield fine particles. Feeding rate was determined to be 3% of body weight, fish were individually weighed weekly. The feeding amount was then recalculated according to weekly weights.

Used *Spirulina*

Blue green algae tablets as food additives, the product information as labelled per three tablets contain 1500 mg, their ingredients are D-Calcium phosphate, microcrystalline cellulose, Anticaking agents (Silicon Dioxide, vegetable

sources Magnesium Stearate). Manufactures in the UK to GMP and pharmaceutical standards, Natures Aid Ltd. Preston PR4 2DQ UK.

Table 1: The Ingredients of experimental diet

Ingredients	Weight in diet (gm/kg)
Fishmeal	150
Soybean	350
Wheat bran	130
Wheat flour	100
Barely Meal	100
Corn Meal	100
Starch	50
Minerals premix	20

Studied traits

Blood was obtained by cutting the caudal peduncle of every fish to use for blood indices and some blood biochemical by using the hematology analyzer BC-2800 which is a compact, fully automatic hematology analyzer with 18 parameters for Complete Blood Count (CBC) test that calibrated for fish blood as the following:

RBC (Red Blood Cell; 10^{12} cells/l), Hb (Hemoglobin; g/l), platelets (10^{9} cells/l), MCV (Mean Corpuscular Volume; fl), MCH (Mean Corpuscular Hemoglobin; pg), MCHC (Mean Corpuscular Hemoglobin Concentration; g/l), WBC (White Blood Cell; 10^{9}cells/l), lymphocytes (%), monocytes (%), granulocytes (%), glucose, total blood protein, lipid profiles (including cholesterol, triglyceride, HDL (High Density Lipoproteins) and LDL (Low Density Lipoproteins).

After dissection liver, gonad and spleen weighted separately to obtain the below traits by using the equation according to (12):

Hepatosomatic index % = liver weight (g)/body weight (g) x 100

Gonadosomatic index (GSI) % = Gonads weight (g)/ Body weight (g) x 100

Spleenosomatic index=Spleen weight/body weight (g)x 100

Statistical analysis

Analysis of variance was conducted by using the general linear models (GLM) procedure of XLSTAT. Pro. 7.5 One way (ANOVA). Fisher's LSD tests was used to compare between means of the control and experiment treatments.

Results and discussion

Table 2 show no significant differences in RBC count, 5 gm *Spirulina* /kg diet in T3 was higher significantly in Hb, control without any *Spirulina* was higher significantly in each of Platelets, MCV, MCH and MCHC.

Adding of *Spirulina* to common carp diets in different levels leads to significant ($P<0.05$) differences in studied differential WBC counts, T3 with 5 gm *Spirulina* /kg diet was higher in WBC counts. Lymphocytes were higher in each of control and T3. Monocytes were higher in control, Granulocytes in T2 with 3 gm *Spirulina* /kg diet, as shown in table 3.

Table 2: Effect of adding different levels of *Spirulina* on some blood indices of common carp *Cyprinus carpio* L.

Treatments	RBC	Hb	PLT	HCT	MCV	MCH	MCHC
T1: 0 *Spirulina*	1.482 A	9.600 B	67.000 A	35.720 B	227.700 A	66.775 A	30.300 A
T2: 3 gm *Spirulina* /kg diet	1.465 A	7.925 C	28.667 C	29.975 C	197.367 B	59.625 AB	29.167 AB
T3: 5 gm *Spirulina* /kg diet	1.796 A	10.700 A	38.000 B	37.200 B	208.567 AB	58.975 AB	27.167 BC

Mean values with different superscripts within a column differ significantly at $P\leq0.05$.

Table 3: Effect of adding different levels of *Spirulina* on WBC differential count of common carp *Cyprinus carpio* L.

Treatments	WBC	lymphocytes	monocytes	granulocytes
T1: 0 *Spirulina*	155.850 B	7.150 A	34.900 A	57.950 B
T2: 3 gm *Spirulina* /kg diet	141.967 C	4.733 B	30.233 C	65.033 A
T3: 5 gm *Spirulina* /kg diet	161.567 A	6.900 A	32.200 B	60.900 B

Mean values with different superscripts within a column differ significantly at $P\leq0.05$.

No significant (P>0.05) differences observed in blood glucose among the treatments, total protein was higher significantly in control and T3 with 5 gm *Spirulina* /kg diet.

Adding of *Spirulina* affected significantly in cholesterol as compared to the control. Triglyceride was higher in control and T2. T3 with 5gm *Spirulina* /kg diet leads to higher and lower significant in HDL and LDL respectively, as shown in table 4.

(13) found significant differences of *Spirulina* on blood parameters of Nile Tilapia fingerlings (*Oreochromis niloticus*). The supplementation of *Spirulina* sp. resulted in no changes of blood parameters or histology of catfish, *Clariasmacro cephalus* x *Clarias gariepinus* in the study of (14).

Table 4: Effect of adding different levels of *Spirulina* on some blood biochemical of common carp *Cyprinus carpio* L.

Treatments	glucose	total protein	cholesterol	triglyceride	HDL	LDL
T1: 0 *Spirulina*	1.735	38.045	2.965	2.600	1.175	1.270
	A	AB	B	A	C	A
T2: 3gm *Spirulina* /kg diet	2.040	36.160	3.385	2.597	1.797	1.070
	A	B	A	A	B	A
T3: 5gm *Spirulina* /kg diet	2.147	38.687	3.250	1.900	3.560	0.377
	A	A	A	B	A	B

Mean values with different superscripts within a column differ significantly at P≤0.05.

According to the results of (15) dietary supplementation of 3.4% *Spirulina* may enhance innate immunity of olive flounder *Paralichthys olivaceus*. They examined the effects of *Spirulina* on serum cholesterol and triglyceride levels, as well as AST and ALT levels, and found significant reductions in cholesterol and triglyceride levels. However, although the levels of AST and ALT were decreased by *Spirulina* supplementation, no significant differences were observed among treatments.

Fish mostly rely on innate immunity in comparison to mammals (16). Accordingly, great attention has been focused on the use of dietary bioactive materials to stimulate innate immunity. The immunomodulatory activity of *Spirulina* has been attributed to its content of C-phycocyanin (17).

A significant difference found in the study of (18) which was conducted to evaluate the effect of replacing fishmeal with *Spirulina* spp. on carcass composition. (8) fed the fish 5% algae had insignificant (P>0.05) increase in total proteins. These findings confirm those reported that the muscle protein of red sea bream was increased as *Ascophyllum* meal supplementation increased, the role of algae on fat content are in full agreement with the finding reported that 5% dietary *Ascophyllum* meal had insignificant increase in muscle fat of red sea bream.

As afore mentioned, overall a lot of factors e.g. levels of *Spirulina*, production system, physiological status and endogenous factors such as nutritional status and composition involved in inconsistent of our results with the other previous reports in fish. Thus, it seems possible to use of *Spirulina* as a protein source in aquaculture industry.

Table 5 showed no significant differences in each of gonadosomatic index and hepatosomatic index, T1 and T3 were higher significantly in spleenosomatic index.

Table 5: Effect of adding different levels of *Spirulina* on some biological parameters of common carp *Cyprinus carpio* L.

Treatments	Gonadosomatic Index	Hepatosomatic Index	Spleenosomatic Index
T1: 0 *Spirulina*	0.774	1.691	0.113
	A	A	AB
T2: 3gm *Spirulina* /kg diet	0.568	2.168	0.068
	A	A	B
T3: 5gm *Spirulina* /kg diet	0.846	1.711	0.119
	A	A	A

Mean values with different superscripts within a column differ significantly at P≤0.05.

The present results agree with the results of (19) which were designed to investigate the effect of different replacement levels of fishmeal with *Spirulina* sp. on growth performance and some blood parameters of common carp *Cyprinus carpio* L., no differences observed among the treatments in the hepatosomatic and gonadosomatic index

and this disagree with the present results. Biochemical analyses often provide vital information for health assessment and management of cultured fish (20,21).

The results indicated that *Spirulina* can be a good choice as an additive for fish diets. Due to supreme level of blood indices involved in fish immunity. The findings of (22) revealed that the dietary addition of microalgae *Chlorella* could increase the immune response. It is noteworthy to say that studies concerning dietary inclusion of *C. vulgaris* as a functional ingredient or as an immunostimulant in koi fish are limited. Furthermore, the *Spirulina* could decrease the level of blood cholesterol, not the glucose of common carp, demonstrating that the *Spirulina* might be involved in the metabolism of lipid. The same results were also found by (22) and (23).

Inclusion of *S. platensis* in fish diet as a feed additive or as a partial replacer of the expensive fishmeal imposes significant promotions in fish growth, coloration, reproduction and flesh quality.

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