

Some Blood Parameters of Common Carp *Cyprinus carpio* L. as Responding To Fishmeal Replacement in Feed with Blue-green Algae, *Spirulina plantesis*

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Abstract

The effect of different levels of replacement in fishmeal feed with Blue-green algae, *Spirulina plantesis* on some blood parameters of common carp, *Cyprinus carpio* was investigated. The trail was conducted for 105 days using 200 fingerlings for common carp with mean weight of (32.7g ± 1.5). Fish were acclimated to laboratory conditions for three weeks and fed with commercial diets (31% protein). Five experimental diets were used and *Spirulina* replaced fishmeal protein within 0% (T1), 5% (T2), 10% (T3), 15% (T4) and 20% (T5). There were significant differences ($P \leq 0.05$) in the White Blood Cell (WBC), Hemoglobin (Hb), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV). In comparison with the control, Red Blood Cell (RBC) count showed insignificant value in comparison with the control. Furthermore the results illustrated that the T5 affect significantly lymphocytes count while the T4 affect the platelets count.

Key words fishmeal, *Spirulina plantesis* and Blood Parameters.

بعض قياسات الدم لأسماك الكارب الشائع *Cyprinus carpio* L. كاستجابة لاستبدال مسحوق السمك في العليقة بالطحلب الاخضر المزرق *Spirulina plantesis*

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الخلاصة

درس تأثير استبدال مستويات مختلفة من مسحوق السمك في عليقة تغذية الاسماك بالطحلب الاخضر المزرق *Spirulina plantesis* في بعض قياسات دم اسماك الكارب الاعتيادي *Cyprinus carpio*. استمرت التجربة لمدة 105 يوم استعملت فيها 200 سمكة بمعدل وزن (32.7غم ± 1.5). اقلمت الاسماك لمدة ثلاثة اسابيع لظروف المختبر وغذيت بالعلف التجاري (نسبة البروتين 31%). استخدم خمس علائق تجريبية واستبدل مسحوق السمك فيها بنسب بالطحلب *S. plantesis* وكالاتي (T1) 0% ، و (T2) 5% و (T3) 10% و (T4) 15% و (T5) 20%. سجلت فروق معنوية ($P \leq 0.05$) لقيم كل من خلايا الدم البيض WBC و الهيموغلوبين Hb ومعدل هيموغلوبين الخلية MCH ومعدل تركيز هيموغلوبين الخلية MCHC ومعدل حجم الخلية MCV باستثناء خلايا الدم الحمر RBC لكل المعاملات مقارنة مع عليقة السيطرة. كما اوضحت النتائج بان المعاملة الخامسة اثرت في تعداد الخلايا للمفاوية بينما المعاملة الرابعة اثرت في تعداد الصفيحات الدموية.

الكلمات المفتاحية: الغذاء الحي للأسماك، *Spirulina plantesis* و قياسات الدم.

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Introduction

The potential use of unconventional foodstuffs such as algae, for substitution the high cost food stuffs such as fishmeal is very important. Algae have attention as a possible alternative protein source for cultured fish, particularly in tropical and subtropical developing countries where algae production rates are high and their higher protein, vitamins and essential fatty acids contents (El-Hindawy, *et al.*, 2006). Microalgae are comprised of all the microscopic organisms, which are suspended in water and included small plants (phytoplankton). The phytoplankton uses inorganic salt, carbon dioxide, water and sunlight, which plays the most important role as the primary base of fish feed web. *Spirulina* is a cyanobacterium (Cyanophyta) that has been commercially cultivated for more than 10 years due to its high nutritional content; e.g. protein, amino acid, vitamin, minerals, essential fatty acid and b- carotene (Vonshak, 1997). *Spirulina* can be considered as nutritional supplement that has various health benefits for humans, and a feed supplement for animals having economic benefits. As an example, it can be a suitable food supplement when fed to trout, sea bass, fancy carp, red tilapia, shrimp and mollusk. It has been found that the alga can be used as an alternative source of protein and can also be used to improve the color, flavor and quality of meat. Nowadays, *Spirulina* can be used to establish immune-potentiating functions in carp (Watanuki *et al.*, 2006; Tongsiri *et al.*, 2010). Mu *et al.*, (2000) and Nandeesh *et al.*, (2001) indicated that *Spirulina* could be used as an effective partially or completely replacement for fishmeal in formulated aqua feeds.

The study of fish blood parameters is important for determining factors related to its physiological capacity Hematocrit, hemoglobin and the erythrocytic hemoglobin concentration values

indicate the oxygen carrying capacity in teleosts. Such parameters are highly varied among the species; they interfere in oxygen-carrying capacity. The blood of all species that have nucleated erythrocytes has the common property of very rapid clotting after it is removed from the body. Among these, the blood of the turtle is probably the slowest to clot, while fish and eel blood are at the other extreme (Affonso, 2001; Wells, *et al.*, 2005; Talal, *et al.*, 2011)

The hematological parameters have been considered as diagnostic indices of pathological condition in animal. Fish blood can serve as a valuable in detecting changes taking place in animal (Talal. *et al.*, 2011).

However, there has been no clear data to indicate whether the effects of *Spirulina* additives for nutrient utilization can be beneficial for growth and whether there is an accumulation of carotenoids in flesh color and stomach content. As a result, the present study should provide information for the preparation of the pellet feed to increase WBC count and differential WBC of the common carp.

Materials and Methods

The objective of this study focused on the effect of different levels of replacement in fishmeal feed with Blue-green algae, *Spirulina plantesis* on some fish blood parameters.

Experimental Diet

Five practical diets were formulated based on the proximate composition of the feed ingredients. Diet 1 (Control diet), diets 2, 3, 4 and 5 contained 5, 10, 15 and 20% dried *S. plantesis* respectively these amount of algae will added as part of fish meal on an equivalent protein basis. Composition and proximate analysis of algae and different experimental diets were shown in tables (1 and 2).

Table (1) The Composition of Experimental Diet

Items	Treatments				
	T1	T2	T3	T4	T5
<i>Spirulina plantesis</i>	0	5	10	15	20
Fishmeal	24.2	21.7	35	35	14.2
Wheat bran	35	35	35	35	35
Soybean	20	20	20	20	20
Broken rice	20.3	17.8	15.3	12.7	10.3
Vitamin	0.5	0.5	0.5	0.5	0.5
Protein %	31	31	31	31	31
Total (100%)	100				

Table (2) The Component of **Spirulina plantesis* Used which Suitable for all Herbivorous Fish such as Pleco's and Catfish as well as Shrimps and Snails.

Composition	Percent
Crude protein	34
Crude Fat & Oils	6
Fiber	5
Ash	10
Vitamin A	24000 IU(per KG)
Vitamin D	2600 IU
Vitamin E	280 IU
Vitamin C	550 mg/kg

*(500g of Premium Sinking *Spirulina plantesis* Wafers, Made in china; Bought from United Kingdom)

Fish and Feeding Regime

Common carp (*Cyprinus carpio*) fingerlings with an average weight of $32.7g \pm 1.5$ were brought from a local aquarium fish supplier located in Al-Kut Governorate Middle of Iraq. Fish were acclimatized in plastic aquaria for three weeks before used in the experiment. Fish were randomly allocated in the aquaria (7/aquarium). Each treatment was represented in four aquaria (4 replicates).

A feeding regime of 3% body weight per day was employed throughout the trail. The amount of food was re-adjusted weekly according to the changes in the fish body weight .

Experimental system

The experimental facility consisted of 20 plastic Aquaria (100 liters each). Each aquarium was supplied with aerated and dechlorinated tap water, which was stored in tanks for 24 hours and aerated by air pump (Model-rina 301) during the experimental period. The water level was maintained to a fixed

level by adding new well-aerated fresh water continually.

Statistical Analysis of Data

Statistical analysis was performed using the Analysis of Variance (ANOVA) two-way classification followed by Duncan's Multiple Range Tests, to determine differences between treatments means at significance rate of $P < 0.05$. The standard errors of the treatment means were also estimated. All statistics were carried out using Statistical Analysis System (SAS) Program.

Results and Discussion

In this experiment, the feed consisted of isonitrogenous and iscaloric feed as was shown in tables (1 and 2).

Table (3) Effect of Replacing Fishmeal with *S. plantesis* on Fish Blood Parameters

Treatment	RBC	WBC	Hb	MCH	MCHC	MCV
T1	0.970 a	175.200 b	122.400 ab	127.400 ab	660.500 ab	197.050 ab
T2	0.860 a	177.350 b	78.500 b	91.100 b	211.500 b	105.900 ab
T3	0.790 a	225.000 a	120.500 a	213.100 a	1118.000 a	212.300 a
T4	0.745 a	183.400 ab	123.500 a	199.000 ab	833.000 ab	89.300 b
T5	0.735 a	223.550 ab	126.500 a	174.100 ab	842.500 ab	206.700 a

RBC (Red Blood Cell: 10^{12} cells/l); WBC (White Blood Cell: 10^9 cells/l); Hb (Hemoglobin: g/l); MCH (Mean Corpuscular Hemoglobin: pg); MCHC (Mean Corpuscular Hemoglobin Concentration: g/l) and MCV (Mean Corpuscular Volume: fl).

Table (4) Effect of Replacing Fishmeal with *Spirulina plantises* on Fish Blood Parameters

Treatments	Gran.	Lymph.	Mon.	PLT
T1	62.300 a	6.600 c	31.100 a	18.000 ab
T2	62.000 a	5.000 c	33.000 a	5.000 b
T3	60.250 a	12.450 ab	33.000 a	7.000 b
T4	54.550 a	7.600 bc	32.150 a	27.500 a
T5	53.050 a	13.650 a	33.300 a	15.000 ab

Gran (Granulocyte: %), Lymph. (Lymphocyte: %), Mon. (Monocyte: %), PLT (Platelet: 10^9 cells / l).

Table (3) showed that fish fed on diets containing 5.0 - 10.0 g *Spirulina*/kg diet exhibited higher RBCs and WBCs counts, glucose, lipids, protein, albumin, and globulin as compared with fish fed on the control diet. These results proved the improvement of fish health when fed *S. plantesis* supplemented diets, in this study, *S. plantesis* could stimulate the immune system via increasing the phagocytic and the natural killer activities (Qureshi and Ali, 1996, Savidov, 2004). Also, Watanuki, *et al.*, (2006) reported that *S. plantesis* activated the functions of leucocytes, such as phagocytosis and production of superoxide, and cytokines production in common carp, *C. carpio*.

The increases in WBCs content recorded in this work when compared with control and other parameters (table 4) could be due to the attempt of the fish to fight against the antigens (pollutant) and this augmented the production of more WBC to improve the health status of the fishes, which agreed with the reports of Venkatesan, *et al.*, (2012)

In the study of Abdel-Tawwab and Ahmad, (2009) who showed that fish fed

a diet containing 5.0 g *S. plantesis* Kg¹ diet exhibited higher RBC and WBC counts, glucose, lipids, protein, albumin and globulin as compared with that fed on the control diet. These results indicate an improvement in fish health when fed *Spirulina*-enriched diets. Moreover, the measurement of albumin, globulin and total protein in serum or plasma is of considerable diagnostic value in fish, as it relates to the general nutritional status as well as the integrity of the vascular system and liver function (Schaperclaus, *et al.*, 1992).

Several authors reported that phagocytosis is stimulated by oral administration of probiotics (Rengpipat, *et. al.*, 2000; Li and Gatlin 2004; Panigrahi, *et al.*, 2005). Moreover, in this study, *Spirulina* could stimulate the immune system (table 4) via increasing the phagocytic and the natural killer activities (Qureshi and Ali 1996). This finding agrees with Duncan and Klesius (1996) who reported that *Spirulina* had the capability of enhancing the non-specific immune responses in fish. However, they demonstrated that peritoneal phagocytes from channel catfish fed *S. plantesis*, showed enhanced phagocytosis to zymosan and increased chemotaxis to *Edwardsiella ictaluri* exoantigen. Neutrophils,

monocytes, and lymphocytes, while the leucocytes with the most positive response to the addition of *S. platensis* to the diet. Feeding *S. platensis* in processed form enhanced specific and non-specific immunity and resistance against *Edwardsiella ictaluri* infection in channel catfish, *Ictalurus punctatus*, (Duncan and Kiesius, 1996). Monocytes and neutrophils, involved in the first line of the defense mechanism and phagocytosis, had the maximum increase among leucocyte types, indicating that *S. platensis* in the diet may benefit the immune system in *X. helleri* as well (James *et al.*, 2006; and Abdel-Tawwab *et al.*, 2008).

In conclusion, our investigation illustrated that the different levels of replacement in fishmeal feed with Blue-green algae, *Spirulina platensis* was play role in fish blood parameters.

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