Impact of dietary supplementation of *Chlorella vulgaris* (Beijerinck, 1890) on the growth, antioxidant defense and immune status of the grey mullet, *Mugil cephalus* (Linnaeus, 1758)

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Abstract

The primary aim of the current study has been to examine the impacts of dietary supplementary of *Chlorella vulgaris* (Beijerinck) powder (CP) different levels on the anti-oxidant enzyme activities, growth, and immune responses of the juvenile *Mugil cephalus* (Linnaeus). Experimental Fish (15 ± 0.1g) was fed diets enriched with 0 (control), 5, 10, and 15 g CP per kg feed for eight weeks period. After the feeding trial period, the fish were challenged against pathogenic bacteria (*Photobacterium damselae* (subsp. piscicida)) for evaluating the resistance of infected fish to diseases. According to the results, fish growth performance was significantly improved with increasing CP levels at CP10 and CP15. Antioxidant-stimulated activity was observed with dietary CP where total antioxidant capacities (TAC), glutathione (GSH), and super-oxide dismutase (SOD) augmented, while malondialdehyde (MDA) decreased significantly in fish fed CP. Furthermore, serum lysozyme activity of fish fed three levels of CP has been considerably enhanced in comparison with the control group ($p < 0.05$). Moreover, CP supplementation at 10 and 15 g dose induced kidney phagocyte and respiratory burst activity which was maximized at 15 g CP. Meanwhile, feeding 10 and 15 g of CP diets decreased mortality in *M. cephalus* after challenge with *P. damselae*. The present study indicated the role of optimal doses *Chlorella vulgaris* extract (10 and 15 g kg^{-1} feed) on growth and antioxidant defense and immune status of grey mullet.

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Keywords: *Chlorella vulgaris. Mugil cephalus.* Kidney phagocytic, Respiratory burst, Lysozyme, Superoxide dismutase (SOD)

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Introduction

It is widely known that aquaculture is the most rapid increasing food production technology throughout the world during over the last 3 decades (FAO 2015). However, there are unfortunately too many challenges for sustainable development of this industry. In intensive aquaculture system massive chemical antibiotics and synthesized drugs usage to protect fish against disease is common while, the danger of excessive use of antibiotics in humans and fish poses a risk by allowing disease microbes to become resistant to antibiotic treatments. As chemical free and organic farming has become very appealing by consumers, scientists started to study the ability of natural immunostimulants to the treat diseases encountered in aquaculture. The most common natural immunostimulants are components of medicinal plant and microorganism like bacteria and microalgae (Awad & Awaad 2017; Defoirdt, Boon, Sorgeloos , Verstraete & Bossier 2007).

Microalgae are valuable sources and have potential as additives to replace by chemical antibiotics that are most commonly used in the animal feed industry (Safi, Zebib, Merah, Pontalier & Vaca-Garcia 2014). Green microalgae, *Chlorella* species have been consumed for thousands of years and in commercially cultivation since 1961. These microalgae are characterized by a growth substance called the *Chlorella* Growth Factor (CGF) composing of free amino acid, peptide, glycoproteins, polyamine, phytohormone, a number of vitamins, minerals, and so on (Doucha 1998). Researchers did a lot of research to evaluate the nutritional value of *Chlorella* as fish feed ingredients. Researchers experimented this microalgae as a feed ingredient or additives in dietary regimes for various experimental fish such as Gibel Carp, *Carassius auratus gibelio* (Bloch) (Zhang Qiu, Xu, Gao, Shao & Qi 2014), Nile tilapia (*Oreochromis niloticus* (Linnaeus)) (Jung, Damusaru, Park, Kim, Seong, Je, Kim & Bai 2017), Olive flounder, *Paralichthys olivaceus* (Temminck & Schlegel) (Rahimnejad, Lee, Park & Choi 2017), juvenile Korean rock-fish, *Sebastes schlegeli* (Hilgendorf) (Bai Koo, Kim & Kim 2001) and rohu, *Labeo rohita* (F. Hamilton) (Andrews, Sahu, Pal & Kumar 2009). The results of these studies showed that different genera of the *Chlorella* improve weight gain and carcass quality of different aquatic animals (Shi, Luo, Chen, Wei, Wu, Zhu & Liu 2017; Kim, Bai, Koo, Wang & Kim 2002; Safi *et al*. 2014; Tibbetts, Mann & Dumas 2017).

Grey mullet, *Mugil cephalus* (Linnaeus) fish is known to feed by grazing the surfaces of aquatic plants. The consumption of this commercial fish species with worldwide distribution is going back centuries. 698,293 tons *Mugilidae* has been totally produced in 2013, of which 80% has been from capture fisheries and 20 % from aquaculture (FAO 2015) and Egypt is the world leader in mullet culture. Scientific information about feeding and immunology parameter of cultured *M. cephalus* is extremely rare. Considering the

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importance of the findings about *C. vulgaris* (Beijerinck), the present research has been done for determining the immune-stimulatory effects of dried powder of microalgae *C. vulgaris* on thjuvenile *M. cephalus.*

Materials and methods

Experimental Diets

The marketed feed has been prepared from Beyza Feed Mill (Iran). Based on the research design, formulations and adjacent chemical analyses of the basal control diets (% as the fed basis) are shown in Table (1). After grinding the feed, different level of *Chlorella vulgaris* powder (CP), 0 (control), 5, 10, and 15 g kg^{-1} of CP were mixed to basal diet. Then, we used a meat grinder for the pelletizing procedure. Afterwards, four experimental diets have been dried and stored at -20 ºC until used (Choi Lee, Nam 2015).

Table 1. Formulation and proximate chemical analysis of the basal control diet (% as fed basis)

Ingredients	**(%)**
Fish meal	30.0
Soybean meal	8.0
Wheat meal	7.0
Squid meal	35.0
Shrimp meal	10.0
Yeast	2.0
Fish oil	1.0
Lecithin	4.0
Vitamins and minerals [a]	2.0
Proximate composition	**(%)**
Protein	46.7
Lipid	16.7
Moisture	9.3
Ash	10.5
Fiber	3.9
Nitrogen free extract	12.7

[a] Vitamins and minerals supplied per Kg. Vitamins: Vitamin A, 2500 U; vitamin D, 2500 U; vitamin E,2000 U. Minerals: 501 mg CuSO4, 1500 mg ZnSO4 ; 0.01 MnSO4 500 CoSO4 ; 500 KI; 35 Na2SeO3.

Fish and rearing condition

We chose Chabahar coast of Oman sea in the south-east of the country, Indian ocean to obtain natural Juvenile grey mullet fish. The fish have been acclimatized to the laboratory condition for two weeks before we start the process of the feeding trials in the Offshore Fisheries Research Center, situated on Chabahar in the south. After adaptation, the juvenile fish (mean 15 ± 0.1 g) have been distributed in a random way to twelve fiberglass tanks so that there have been 30 fish in each tank. Then, the experimental diets have been used to feed the fish. After feeding, three replicate groups of the fish have been fed by hands to a clear repletion 3 times per day (0800, 1300, and 1700 for six days/weeks) for eight weeks. Moreover, we measured the parameters of water quality daily. Then, we kept water at 28.5 °C, dissolved oxygen 7.81 ± 0.3 mg L^{-1}, salinity 33.2 ± 0.35 g L^{-1}, and pH 7.8 ± 0.2. Afterwards, we fed the fish with diets of different dose of CP including 0, 5, 10 and 15 g kg^{-1}. When eight weeks elapsed from the experiments, bacteria *P. damselae* has been chosed to infect ten fish from each dietary treatment for ten days for evaluating the resistance of the infected fish to diseases.

Growth parameters assay

We used the early and resulting weights of the entire fish in all groups and alterations for calculating the growth performances.

The above procedure is presented here:

WG, refereeing to the weight gain = [(final body weight - initial body weight) / initial body weigh] × 100

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SGR, representing the specific growth rate = [(ln final body weight - ln initial body weight) / days] × 100

FCR, representing the feed conversion ratio = Wet weight gain × 100 / feed intake

Total anti-oxidant capacity and antioxidant enzyme activities

In order to continue our tests, the liver tissue has been dissected. Ice-cold 0.9% NaCl has been used to fully wash it. Then, we weighed, cut up, and homogenized (10% w/v) them via 100 mM phosphate buffer, 100 mM KCK, 1 mM EDTA with Ph = 7.4. Afterwards, the homogenate, which has been centrifuged at 1,000 g at 4 °C for twenty minutes, has been employed for assaying TAC and estimating SOD, MDA, and GSH of the tissue of the fish liver (Atli & Canli, 2010.). TAC has been gauged by a commercial kit according to the company's instructions (ZellBio GmbH; Germany) with regard to the oxidation reduction colorimetric assays at a wave-length of 490 nm. Based on our research, TAC levels have been regarded as the amounts of the anti-oxidant in the sample, which has been contrasted with ascorbic acid action as the standard. The mentioned technique may determine TAC with 0.1 mM sensitivity (100 μmol L^{-1}). Researchers argued that intra-assay and inter-assay variation coefficient is <3.4% and 4.2%. Thus, SOD activity unit has been regarded as the amounts of the samples, which catalyzed decomposing1 mol of O_2^- into H_2O_2 and O_2 in each minute. Adsorbance has been registered at 550 nm. Levels of MDA have been measured via commercial chemical colorimetrical assay kit based on the company's directions (MDA assay kit: ZellBio GmbH, Ulm, Germany). It should be noted that MDA applies the MDA-TBA adduct created via reacting MDA and thio-barbituric acid (TBA) at high temperature. Acidic media has been used to measure MDA. Then, it has been heated (90-100 °C) colorimetrically at 535 nm. Using this technique, MDA can be determined with 0.1 μM sensitivity. Then, intra-assay and inter-assay coefficients of variations have been 5.8% and 7.6%. GSH assay has been measured with the marketed chemical colorimetric assay kits (ZellBio GmbH, Ulm, Germany) via a colorimetrical procedure at 412 nm. It has been found that the assay sensitivity equals 0.1 mM. Moreover, the intra-assay and inter-assay coefficients of variations have been 6.1% and 7.7%. Bradford technique (1976) has been used to measure the total soluble protein via the bovine serum albumin as a standard. Each enzymatic assay has been implemented in triplicate (Bradford, 1976).

Collection of macrophages from head kidneys

According to our research plan, we cut the head kidneys of the fish, transported them directly to the Leibovitz L-15 culture medium (where pH has been set at 7.8) (Merck: Germany). Based on the processes illustrated by Kim and Austin (2006), individual separation of the head kidneys has been done via disruption across a nylon mesh (100μm) with L-15 medium with 2% (v/v) fetal calf serum (FCS), 10 μl mL^{-1} heparin, and 100 μl mL^{-1} gentamycin. Final suspension has been layered consistently on to a 34 to 51% (v/v) Percoll gradient diluted in the Hank's Balanced Salt Solution (HBSS, Sigma)

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prior to the centrifugation of tubes at 400 × g at 4 °C for 25 minutes. The cells band situated on the 34% to 51% interface has been gathered. Then, HBSS has been used to wash it two times. Density of the cell has been set at 10^6 cells ml^{-1} in L-15 medium complemented with 0.1 % (v/v) FCS and 100 µl mL^{-1} gentamycin. Trypan blue exclusion technique has been used to evaluate the viability (Kim & Austin, 2006).

Respiratory burst activity assay

After doing the above procedures, quantification of the respiratory burst activities of the separated head kidneys of the fish has been done through nitroblue tetrazolium (NBT) assay (Secombes, 1990). The assay deals with the measurement of the quantities of intra-cellular oxidative free radicals (Ardó, Yin, Xu, Váradi, Szigeti, Jeney & Jeney 2008).

Phagocytosis assay

Phagocytic activities of the macrophages of the head kidney have been specified. To sum up, 1 ml of the macrophage cell suspension (10^6 cells ml^{-1}) that has been achieved from each individual fish has been adhered on to a methanol-cleaned glass slide at 18 °C in a moist space for one hour. In order to remove nonadherent cells, HBSS has been used to wash it prior to the addition of 1.0 ml auto-claved congo red-colored yeast cells (10^8 cells mL^{-1}). Of course, we let phagocytosis to continue for one hour. Then, the air-dried slides have been fixed in absolute methanol for three minutes, and staining has been done via Giemsa's technique for fifteen minutes. About 200 cells have been randomly counted, and PA has been written as:

PA = the number of the phagocytosing cells/numbers of total cells × 100

Numbers of the yeast cells phagocytosed in each macrophage cell have been used to determine the phagocytic indicator.

Lysozyme assay

Random sampling of 3 fish from each group has been taken when the test ended. About 2 cc blood has been gathered from the fish caudal vein, and anti-coagulant heparin has been added. Then, centrifugation of the blood samples has been done at 3000 g for five min, and plasma has been kept at -80 ^oC so that it can be employed for assaying the plasma lysozyme.

According to Parry, Chandan, and Shahani (1965), turbidimetric assay for lysozyme has been done with partial modifications (Ellis, 1990). To sum up, substrate for assaying the lysozyme has been conducted by 0.03% of the lyophilized cells of *Micrococcus lysodeikticus* (Sigma; ATCC No.: 4698) in a buffer of 0.05 mM sodium phosphate (pH = 6.2). Then, 25 µl of the fish plasma have been added into 175 µl bacterial suspension of the duplicate wells of a microtitre plate. Incubation of the mix has been done at room temperature. Afterwards, adsorbance at 600 nm has been gauged after 15 seconds through an ELISA plate reader (Argus; PerkinElmer: France). Each unit of lysozyme activities has been described as a plasma-decreasing amounts of lysozyme in the adsorbance of 0.001 mL^{-1} min^{-1}.

Challenge test with *P. damselae*

When the fish have been fed for eight weeks, we began the challenge stage. This stage has been done on all experimental groups with

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SK7 strain of *P. damselae* primarily separated from the suspected fish. However, Iran Veterinary Organization (IVO) in Chabahar has been chosen to do so. Then, 10 mL of the liquid brain heart infusion broth (BHI; Sigma) media have been applied to inoculate the bacteria. Afterwards, the bacteria have been grown at 30 °C for 24 to 48 hours. Next, their centrifugation has been done at 850 g for fifteen minutes. Then, we removed the supernatant. Sterile phosphate buffered saline (PBS) solution has been used to wash the pelleted bacteria two times. Afterwards, the bacteria concentrations have been set at LD70 = 7.2×10^4 through the suspension optical density (Austin & Austin 2007). At the end, incubation has been done into an aquarium water. Then, the fish have been submerged into it for four hour. Additionally, over a 10-days challenge test, the data for cumulative death have been registered.

Statistical analyses

Normality of the data has been examined by Kolmogorov–Smirnov statistic. Moreover, the variances homogeneity has been checked before we compare them. SPSS22 (Armonk, NY, USA) and one-way ANOVA have been used to analyze the data. One-way ANOVA, which performs mean comparison with Duncan's test at a reliability level of 0.05, has been used to analyze the statistical differences among mean values with independent variables.

Results

Growth performance

As seen in Table 2, the growth performance of the juvenile grey mullet, *M. cephalus, which have been* fed by 4 experimental dietary regimes lasted for eight weeks. This research revealed that the fish specific growth rate (SGR), feed conversion ratio (FCR), and the weight gain (WG), which had been fed dietary regimes with CP 10 and CP 15, significantly increased than those fed with CP 5 and CP 0 diets ($P < 0.05$).

Table 2. The growth performance of the grey mullet fed with experimental dietary regimes with various amounts of CP for eight weeks

Parameters	Experimental diets CP (g kg^{-1})			
	0	5	10	15
Initial body weight (g fish^{-1})	15.0 ± 0.12	14.9 ± 0.09	15.0 ± 0.11	15.0 ± 0.11
Weight gain (%)	202.9 ± 10.26^b	208.4 ± 18^b	222.5 ± 16^a	219.1 ± 19^a
Specific growth rate (%)	1.68 ± 0.21^b	1.80 ± 0.17^b	1.95 ± 0.12^a	1.98 ± 0.14^a
Feed conversion ratio (%)	1.25 ± 0.01^b	1.22 ± 0.01^b	1.01 ± 0.00^a	1.05 ± 0.01^a

Values (means ± SE, n =3) with various superscripts in the same row have significant difference ($P < 0.05$).ns-values are not significant ($P > 0.05$).

Activities of the antioxidant enzyme

Table 3 shows the liver TAC and its tissue antioxidant enzyme activities such as SOD and GSH of the grey mullet fish. Dietary intake of CP significantly enhanced TAC content of fish at CP 10 and CP 15 levels ($P < 0.05$). After 8 weeks, we saw an increasing trend in the activities of the SOD and GSH of the fish who

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have been completely fed from CP0 to CP 15 dosage ($P < 0.05$). MDA levels of the fish given feed with a diet containing 10 and 15 g CP were lower than control and to 5 g CP ($P < 0.05$).

Table 3. Overall anti-oxidant capacities and anti-oxidant enzyme activities of the grey mullet fed with the experimental dietary regimes with various amounts of CP for eight weeks

Anti-oxidant enzyme (U mL^{-1})	Experimental diets CP (g kg^{-1})			
	0	5	10	15
TAC	5.67 ± 0.39 ^{c}	5.85 ± 0.43 ^{c}	8.89 ± 0.33 ^{b}	9.73 ± 0.34 ^{a}
SOD	2.49 ± 0.27 ^{c}	3.22 ± 0.17 ^{b}	4.04 ± 0.18 ^{ab}	4.73 ± 0.42 ^{a}
GSH	1.52 ± 0.03 ^{d}	2.27 ± 0.15 ^{c}	3.23 ± 0.18 ^{b}	3.93 ± 0.06 ^{a}
MDA	53.84 ± 2.42^{a}	53.25 ± 1.98^{a}	34.09 ± 1.23^{b}	33.44 ± 1.27^{b}

Values (means ± SE, n = 3) with various superscripts into the same row have significant difference ($P < 0.05$). ns-values are not significant ($P > 0.05$).

Activities of lysozyme, phagocytic and respiratory burst

Table 4 presents the activities displayed by lysozyme, phagocytic, and respiratory burst. According to the results, the lysozyme activity of the fish fed with CP at three level have been remarkably increased as compared with the ones fed with control diets ($P<0.05$). Moreover, CP dietary regimes applied stimulatory impacts on the phagocytic activities of the head kidney macrophages of grey mullet fish. It should be noted that NBT has been reduced by by CP 10 and CP 15 concentration diets. Nevertheless, responses caused by CP 15 considerably increased compared to the CP 10 ($P<0.05$). Moreover, the respiratory burst activities of the head kidney macrophages have been in the range between 0.67 and 0.81 optical density having minimum CP 0 and CP 5 diets and maxumum diet CP15.

Table 4. Analyses of the immune factors of grey mullet fed with experimental dietary regimes with various amounts of CP for 8 weeks

Immune parameter	Experimental diets CP (g kg^{-1})			
	0	5	10	15
Lysozyme activities (U mL^{-1})	149.0 ± 2.64 ^{d}	160.33 ± 5.6^{c}	182.16 ± 3.7 ^{b}	192.67 ± 4.18 ^{a}
Kidney respiratory Burst activities (620 nm)	0.67 ± 0.02 ^{c}	0.69 ± 0.01 ^{c}	0.74 ± 0.03 ^{b}	0.81 ± 0.01 ^{a}
Kidney phagocytic activity (UmL^{-1})	44.0 ± 1.01 ^{b}	45.26 ± 0.64 ^{b}	50.22 ± 0.69 ^{a}	50.55 ± 0.59 ^{a}

Values (means ± SE, n = 3) with distinct superscripts into the same row have significant difference ($P < 0.05$).

Resistance to the disease of the grey mullet M. cephalus exposed to the bacterial infections with P. damselae

The rate of the death has been remarkably declined in comparison with the controls, which have been supplemented diet with 10 and 15 g concentrations of CP (Figure 1). According to Figure 1, CP0 has shown the maximum cumulative percent of the death with 79.0 ± 5.0 % over a ten-day exposure to the lethal dosage of *P. damselae*. Total rate of the death for CP0 group ranged between 8.0 ± 1.0% at the 1st day and 79.0 ± 5.0 % at day 10 (Figure 1). When inoculating has been accomplished for ten days, minimum total percent of the death has been

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found in CP15 supplemented diet reached to 23.0 ± 3.0 %. This was followed by 25.0 ± 2.0 % for CP10 diet and 70.0 ± 5.0 % for CP5 (Figure 1).

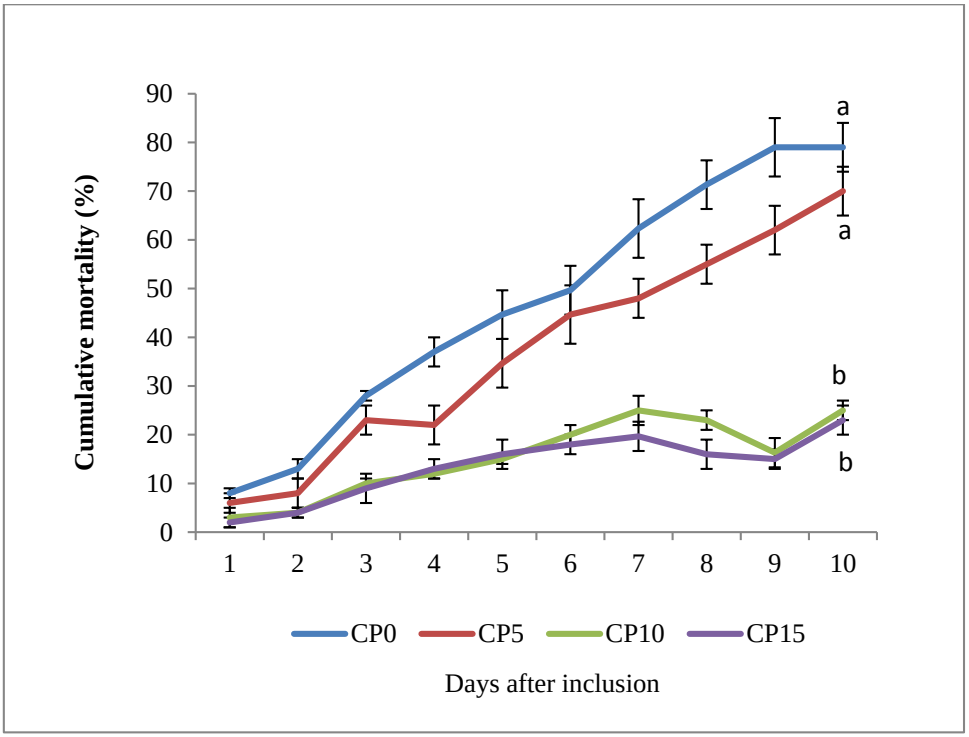


Figure 1. Total death (%) of the grey mullet fed the experimental diets composing of various levels of CP for eight weeks and consequently challenged with *P. damselae.*

Discussion

Chlorella species are popularly used in rearing of fishes at different growth stages. This is the first study that deals with the evaluation of the inclusion of dietary *C. vulgaris* on the antioxidant and immune responses and growth of the juvenile grey mullet. The present study indicated that diet enriched with CP at 10 and 15g kg^{-1} could enhance weight gain and SGR in *M. cephalus*. The review of previous study showed that *Chlorella* as an main ingredient or additive have been tested for several fish such as Korean rock-fish, *Sebastes schlegeli* (Bai *et al.*, 2001), crucian carp, *Carassius auratus* (Xu, Gao, Qi, Qiu, Pengq & Shao 2014), Nile tilapia, *Oreochromis niloticus* (Badwy Ibrahim & Zeinhom 2008), olive flounder, *Paralichthys olivaceus* (Rahimnejad *et al*., 2017), Koi, *Cyprinus carpio* (Linnaeus) (Khani, Soltani, Shamsaie Mehrjan, Foroudi & Ghaeni 2017). The optimum dietary CP supplementation dose for positive effects on growth and feed utilization of Korean rockfish was 5g kg^{-1} versus to 15g kg^{-1} for *M. cephalus* in current study. This difference might be due to different ability of fish to utilize microalgae. Grey mullets have often been characterized as detritus and deposit feeders and they can also exploit plankton and other suspended organic matter. No other group of fish relies so much on plankton. Therefore, it seems grey mullets can tolerate much of microalgae in their diet as source of energy to improve their growth

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performance. Studies reported that dietary inclusion 10–15% of algae meal are suitable and more efficiently for fishes with more amylase activity (herbivorous and / or omnivorous species, such as common carp (*Cyprinus carpio*) (Diler, Tekinay, Guroy, Guroy & Soyuturk 2007) and Nile tilapia (Ergün, Soyutürk, Güroy, Güroy & Merrifield 2009).

Chlorella species have been screened for their possible radical scavenging activity and many of these species exhibited potent in-vivo and or in-vitro anti-oxidant activity (Becker 2004). Oxidative stress is a major cause of a large number of diseases. In the present study, total antioxidant capacity and antioxidant enzymes including SOD and GSH tended to have positive relationship by the inclusion of 10 and 15 g of CP. According to the review, these findings have consistency with other studies conducted in olive flounder, *Paralichthys olivaceus* (Rahimnejad *et al*., 2017) and gibel carp (Xu *et al*., 2014). Their results showed that including dietary *Chlorella* affects positively the anti-oxidant enzyme of experimental fish. It should be noted that the level of enzyme activity varies with species and tissue type.

The variations in antioxidant potential of *Chlorella* and its impacts on the fish antioxidant capacity could be ascribed to amount of polyphenolic and phenolic compounds of this microalga (Andrews *et al*. 2009; Badwy *et al.*, 2008). Based on our experience it seems antioxidant activities of algae diet are seriously influenced by the drying procedure prior to using them. Drying methods including shadow, sun, freeze or oven drying resulted in a difference in the total polyphenol content and consequently it can changes their usefulness in fish antioxidant defense.

In order to replace natural immunostimulants to chemical and synthetized antibiotic, the potential of algae and algal derivatives have been studied as an additive of the diet for enhancing the disease resistance in cultured fish. Moreover, the immune-stimulant effects of dietary supplements in the fish have emphasized primarily on the assessment of the non-specific immune factors. In current study inclusion *Chlorella* powder from 5 to 15 g improved serum lysozymes and kidney respiratory burst activities in grey mullet. It is widely known that lysozyme is one of the significant defense enzyme and key agent for evaluation of innate immune response in fish. Its variation is related to production activated macrophages, leucocytes and macrophages. The increased lysozyme activity in present study indicated that CP can be suitable immunostimulant for grey mullet fish.

So far, a majority of the assessments reported on the bio-activity of the algal food have been done in the short-term in-vitro experiments, so clear information of the behaviors of the algal food into the body of fish are lacking. In this study, different concentration of CP was added in the basal diet of fish. Results showed that administration 10 and 15 g of CP caused considerable enhancement of phagocytic and respiratory burst activity in grey mullet fish. The achieved findings agree well with the findings published by Zhang et al., 2014. Zhang et al.'s study revealed possible involvement of the dietary

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Chlorella in the regulation of the adaptive and innate immunities of gibel carp, *C. auratus gibelio*. Scientists believe that the activity of *C.vulgaris* is associated with Chlorella Growth Factor (CGF) which consists of peptide, glycoprotein, free amino acid, polyamine, phytohormone, a number of vitamins and minerals. It should be noted that, their mechanism would be proceeded by modulating the production of cytokine (Hasegawa, Yoshikai, Okuda & Nomoto 1990; Morris, Carrillo, Almarales, Bermúdez, Lebeque, Fontaine, Llauradó & Beltrán 2007).

P. damselae is an emerging pathogen bacterium that causes infections and fatal disease in aquaculture. As shown in the current research, natural hosts of the pathogen are different kinds of marine fish and it has significant economic impacts on wild and farmed marine fish worldwide. Researchers also found that the juveniles and larvae have higher susceptibility to *photo-bacteriosis* (Andreoni & Magnani, 2014). With regard to the outputs, the supplementary diets composing of different concentrations of CP highly declined death in comparison with the controls. Moreover, dosing CP 15 has been the most appropriate does for treating *M. cephalus* versus bacteria *P. damselae*. The previous research has strongly emphasized the algal diets reduce mortality against pathogenic challenges in various animals. For example higher resistance of the rats to *Escherichia coli* infections in a group, which had been underwent oral administration with *C. vulgaris* extract (Hasegawa *et al*., 1990). As microalgae *C. vlgaris* currently are produced by commercial company in large scale, replacement of chemical antibiotic by this natural immunestimulant source can help sustainable aquaculture and specially help grey mullet farms to produce safe and healthy production.

Lastly, this research represented the improvement of growth, antioxidant defense, and immune responses in the juvenile grey mullet fish by the dietary supplement of 15 g *Chlorella* powder as the feed additive.

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Conflict of interests

The authors declare that there is no conflict of interest.

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تأثیر مکمل غذایی کلرلا (*Chlorella vulgaris*) بر رشد، دفاع آنتی اکسیدانی و وضعیت ایمنی ماهی کفال خاکستری (*Mugil cephalus*)

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چکیده

هدف اصلی از مطالعه حاضر، بررسی اثر سطوح مختلف مکمل غذایی پودر کلرلا (*Chlorella vulgaris*) روی رشد، فعالیت آنتی اکسیدانی و پاسخ ایمنی ماهی کفال خاکستری (*Mugil cephalus*) جوان می‌باشد. ماهی‌های مورد آزمایش (۰/۱ ± ۱۵ گرم) با رژیم های غنی شده با ۰ (شاهد)، ۵، ۱۰ و ۱۵ گرم پودر کلرلا بر کیلوگرم غذا برای یک دوره ۸ هفته‌ای تغذیه شدند. بعد از دوره تغذیه، برای ارزیابی مقاومت ماهی‌ها در مقابل عفونت باکتریایی، ماهی‌ها با باکتری فتوباکتریوم دمسلا (*Photobacterium damselae*) چالش داده شدند. بر طبق نتایج، با عملکرد رشد ماهی با افزایش میزان پودر کلرلا (۱۰ . ۱۵ گرم بر کیلوگرم غذا) به طور مؤثر بهبود یافت. تحریک فعالیت آنتی اکسیدانی در رژیم های غذایی حاوی پودر کلرلا مشاهده شد به طوری که میزان آنتی اکسیدان کل، سوپر اکسید دیسموتاز و گلوتاتیون در ماهیان تغذیه شده با پودر کلرلا افزایش یافت در حالی که مالون دی آلدئید کاهش معنی‌داری را نشان داد. همچنین میزان لیزوزیم سرم در ماهیان تغذیه شده با سطوح پودر کلرلا افزایش معنی‌داری را در مقایسه با گروه شاهد نشان داد ($P<۰/۰۵$). به علاوه استفاده از ۱۰ و ۱۵ گرم مکمل کلرلا بر کیلوگرم غذا فعالیت فاگوسیتوز کلیه و انفجار تنفسی را تحریک کردند و بیشترین میزان این فعالیت‌ها در سطح ۱۵ گرم مکمل کلرلا بر کیلوگرم غذا مشاهده شد. تغذیه با ۱۰ و ۱۵ گرم مکمل کلرلا بر کیلوگرم غذا مرگ و میر کفال ماهیان بعد از چالش با باکتری فتوباکتریوم دمسلا را کاهش داد. مطالعه حاضر نقش مؤثر دوزهای بهینه ۱۰ و ۱۵ گرم پودر کلرلا بر کیلوگرم غذا بر رشد، دفاع آنتی اکسیدانی و وضعیت ایمنی ماهی کفال خاکستری را نشان داد.

کلمات کلیدی: کلرلا ولگاریس، ماهی کفال خاکستری، فاگوسیتوز کلیه، انفجار تنفسی، لیزوزیم، سوپر اکسید دیسموتاز

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