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Effects of Dietary Macroalgae *Gracilaria pygmaea* on Asian Sea Bass (*Lates calcarifer*) Juveniles

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ABSTRACT

Asian sea bass (*Lates calcarifer*) an important marine species of high economic value and excellent meat quality, has suffered great losses due to disease in high-density aquaculture. The macroalgae, *Gracilaria pygmaea*, which include various bioactive compounds, may serve as an immunostimulant in the aquaculture industry. This study aimed to assess the effect of dietary fish meal replacement with *Gracilaria pygmaea* meal on immunity, activity of liver antioxidant enzymes, intestinal tissue, *lysozyme* gene activity, and *IGF-I* gene activity in Asian sea bass (*Lates calcarifer*). To this end, 120 individuals of Asian sea bass with an average weight of 28±0.5 g were divided into four treatments and three replicates and kept in 12 tanks (10 fish per 300-liter tank). Blends of soybean meal and fish meal were used as a Control diet (C). Experimental diets were prepared to substitute the fish meal with 3 (GL3), 6 (GL6), and 9% (GL9) of *G. pygmaea* in the basal diet. Fish were fed three times daily for six weeks. Dietary supplementation of *G. pygmaea* led to significantly increase in total immunoglobulin level compared to the control. The inclusion of *G. pygmaea* in the diet did not affect the antioxidant status of the fish. The histological analysis showed that fish of all groups exhibited normal morphology of anterior intestine and pyloric caeca. The obtained results showed that fish of GL9 and the control groups had the highest IGF-1 mRNA transcript abundance in the liver compared to the other groups. The changes of lysozyme expression noticed among the groups were statistically insignificant. Overall, the results obtained in this study indicated that dietary *G. pygmaea* did not cause adverse effects on immune status, antioxidant status, intestinal morphology, and *lysozyme* gene activity in Asian sea bass.

Keywords: Antioxidant status, Fish, Immune response, Histology

INTRODUCTION

Presently, aquaculture is the source of half of the world fisheries production for human use and around 8–9% of the animal protein consumption. It is envisaged that aquaculture will supply more than 60% of fish used for human direct use in 2030 (FAO, 2014). Over the next 40 years, human population is increasing to more than 10 Billion. Demand for fisheries products will increase in the future; therefore, aquaculture will have to increase (Davis, 2015). The ingredients providing proteins are usually the most expensive; so, the prices of fish have steadily increased in recent years due to increasing demand and limited supply. Therefore, global markets look for an appropriate alternative source (Arruda *et al*., 2007). Terrestrial plant-based sources that are used to replace fishmeal usually contain fiber (Opstvedt *et*

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al., 2003) and a variety of anti-nutritional factors or toxicants (Krogdahl *et al*., 1994; Francis *et al*., 2001), as well as general nutritional misbalanced essential amino acids (Floreto *et al*., 2000).

Therefore, increasing these ingredients can decrease nutrient digestibility, overall growth, and feed efficiency in some species (Refstie *et al*., 1998; Chou *et al*., 2004). In addition to ongoing studies on the application of protein sources in drought plants, which replaced the fishmeal with dietary fish, humans have also sought new sources, and nowadays, seaweed is a good candidate for these protein sources. Seaweed can provide better-balanced nutrition and improve overall animal growth because it contains essential fatty acids, pigments, antioxidants, and polysaccharide components (Rajapakse *et al*., 2011). Improved growth, dietary intake, liver function, lipid metabolism, physiological activity, response to stress, disease resistance, and meat quality have been reported in rations containing 1-5% algae (Nakagawa *et al*., 2007). According to a review by Holdt and Kraan (2011), seaweed polysaccharides, such as carrageenan, alginates, β-glucans, and sodium alginate show great stimulatory effects on immunity, and protect fish against microbial diseases (Castro *et al*., 2005; Fujiki *et al*., 1994; Gabrielsen *et al*., 1998). The main polysaccharide in *Gracilaria* spp. is agar with similar structural and functional proprieties to carrageenan (Araújo *et al*. 2016). Agar enhanced the non-specific immune response of basa (*Pangasius bocourti*) against *Aeromonas hydrophila* (Van Doan *et al*., 2014). It appears that low molecular weight polysaccharides derived from agar bearing seaweeds are fermented by gut bacteria and can be a source of prebiotics (Ramnani *et al*., 2012). *Gracilaria* algae belong to the Gracilariaceae family (Rhodophyta). *Gracilaria pygmaea* species is available on the Persian Gulf coast in vast amounts throughout the tidal zone, due to its high production in the wild and artificial production. This species can be considered a food source for aquatic animals by aquatic food manufacturers. Xu (2011) reported that *S. canaliculatus* fed *Gracilaria* spp. supplemented diets significantly increases lysozyme activity. Van Doan (2014) reported that *Pangasius bocurti* fed agar supplemented diets significantly increases lysozyme activity.

Asian sea bass (*Lates calcarifer*), also known as barramundi, is an indigenous species in the Indian region and in the Pacific Ocean, and is scattered in the waters of the Persian Gulf to Australia (Glencross, 2006). Asian sea bass has a high degree of farming potential because of its excellent meat quality, adaptive capacity, and capability to adapt to varying degrees of salinity (Boonyaratpalin *et al*., 1998; Singh, 2000).

In the current study, we aimed to partly replace the fish meal with red seaweed (*Gracilaria pygmaea*) to evaluate this alga as a fishmeal substitute and assess its effect on immunological status, activity of digestive antioxidant enzymes, intestinal morphology, and gene expression level in Asian sea bass (*Lates calcarifer*) juveniles.

MATERIALS AND METHODS

Experimental Design

The Asian sea bass (*Lates calcarifer*) were purchased from Ramooz Company (Bushehr, Iran) and transported to the laboratory of the Aquatic Research, Persian Gulf University. One hundred twenty piece fish were acclimated to laboratory conditions for two weeks in two 4,000-L tanks and fed on a commercial diet (Byza, Iran) containing 47% crude protein, 17% crude fat, 2% crude fiber, and 14% ash, before starting the experiment. After the adaptation phase, fish with an average weight of 28±0.5 g were randomly selected and stocked in twelve 300-L tanks (triplicate groups per dietary treatment) at ten fish per tank density. They were fed with dietary

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supplements for six weeks, twice a day (at 10_{am} and 5_{pm}) for 40 days (Azodi *et al*., 2016). *Gracilaria pygmaea* was collected from the Persian Gulf coast area, spread out after washing in the tray (extending the surface to dry), and dried in shade for 24 hours. Then, the product was collected and dried in a furnace at 60°C for 24 hours and then milled. The basal diet was formulated, containing 46% crude protein and 18% crude lipid (Table 1). This diet satisfied the crude protein and crude lipid provisions of Asian sea bass (NRC, 2011). The other three test diets were formulated by adding the increasing levels of *G. pygmaea* to the basal diets. Fishmeal was replaced at levels of 3 (GL3), 6 (GL6), and 9% (GL9) with dried *G. pygmaea*, and a positive control test diet was free of algae (basal diet). After determining the percentage of the required food components, the required materials first passed through a 500-micron sieve (< 1 mm). All ingredients were mixed with fish oil and soybean oil. Then, water was added to produce a dough and components of food were mixed for 15 minutes to obtain a pellet with an average diameter of 3 mm. The moist pellets were dried in a forced air oven at 60°C for about 12 hours, then, stored at -20°C until used (Nafisi *et al*., 2008). The formulation and chemical composition of the experimental diets are shown in Table 1.

The physicochemical parameters of water containing temperature, dissolved oxygen, pH, and salinity were measured (WTW model B3223/set 1), during the test period daily. The light period was 12 hours of light and 12 hours of darkness. All seawater used during the rearing process was collected from the Persian Gulf and was filtered for use.

Immune Analysis

Four fish per tank (12 per treatment) were randomly sampled and anesthetized (2-

Table 1. Ingredients and chemical composition of the experimental diets (g/kg).

Ingredient	Diets			
	C	GL3	GL6	GL9
Fish meal[a]	54	42.68	41.36	40.04
Soybean meal[b]	0	13.62	13.64	12.86
Wheat gluten	11.9	12	11.8	11.9
Wheat meal	10.6	4.5	3	2
Fish oil	6.4	6.75	6.75	6.75
DGP[c]	0	3	6	9
Soybean oil[d]	6.4	6.75	6.75	6.75
Vitamin premix[e]	1.5	1.5	1.5	1.5
Mineral premix[f]	1.5	1.5	1.5	1.5
Squid meal	1.5	1.5	1.5	1.5
Antioxidant	0.2	0.2	0.2	0.2
Gelatin	5	5	5	5
Chromium oxide ($cr_2 o_3$)	1	1	1	1
Proximate analysis (% dry diet)				
Crude protein	46.26	46.67	46.9	46.57
Crude fat	18	18	18	18
Ash	16	15.65	16.47	16.78
Moisture	11	11	11	11

[a] Pars Kilka (Mazandaran, Iran)- 63.5 crude protein, 17.7 crude lipid); [b] Havorash (Bushehr, Iran)- 44.5 crude protein, 1.5 crude lipid); [c] Dried *Gracilaria pygmaea*- Moisture, 8.1; protein, 16.68; lipid, 1; fiber, 1.2; ash, 29.5 (% dry matter); [d] Product of Kesht Va Sanat Shomal Vegetable oil Factories Complex (Neca, Iran); [e] Vitamin and mineral premix (supplied by Beyza Feed Mill, Fars, Iran) and covered known requirements for Asian sea bass; [f] Nitrogen-Free Extracts (NFE) = 100-(Crude protein+Crude lipid+Fiber+Ash).

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phenoxyethanol at 0.5 mL L^{-1}) for blood sampling and serum analysis at the end of the feeding trial. The whole blood was collected in a syringe, allowed to clot in microtubes at room temperature, and stored in a refrigerator (4 hours at 4°C). Serum was harvested by centrifuging at 1,600×g for 5 minutes at 4°C and preserved at −20°C. Serum lysozyme activity was determined by the method described by Kim and Austin (2006) based on the lysis of the lysozyme- sensitive gram-positive bacterium *Micrococcus lysodeikticus* (Sigma, USA); the results of lysozyme activity are given as units per mL. The serum total immunoglobulin level was measured according to Siwicki *et al*. (1994). The complement replacement activity was measured using Rabbit Red Blood Cell hemolysis (RaRBC) according to Yano (1992).

Intestinal Histology

At the end of the feeding experiment, two fish in the treatment group were taken for histological examination, and fixed in phosphate-buffered formalin 10%. The tissue samples were processed according to the protocols described by Roberts (2001) and sectioned using a microtome Leica RM 2245 (Leica Biosystems, Nussloch, GmbH, Germany). Photomicrographs of the intestine were recorded using an Olympus CX41 microscope with a digital camera C7070 attachment.

Antioxidant Enzyme Measurement

Glutathiones-S-Transferase (GST) (EC 2.5.1.18) was determined by absorbance at 340 nm, using 1-Chloro-2,4-dinitrobenzene as substrate, consistent with the methods described in Habig *et al*. (1974). Glutathione Reductase (GR) (EC1.8.1.7) was evaluated based on NADPH (Sigma, Portugal) oxidation at 340 nm (Mc Farlan *et al*., 1999).

Evaluation of Relative mRNA Transcript Abundance of Growth and Immune-Related Genes in the Liver

Total RNA in the liver was extracted by a high pure RNA tissue kit (Roche, Manheim, Germany) under the manufacturer's instructions. Then, the concentration of RNA was evaluated spectrophotometrically at 260 and 280 nm, and samples with the RNA ratios (A260:A280) higher than 1.8 were chosen following electrophoresis on a 1% agarose gel. Elongation factor 1 α (*Ef1a*) was applied for the internal housekeeping gene. For synthesizing the first-strand cDNA, a transcription Kit (Robinteb, Tehran, Iran) was used according to primer sequences Reverse Transcription (RT) by using one μg of total RNA with the Random Hexamers and M-MuLV Reverse Transcriptase enzyme kit (Vivantis) following the protocol provided by the manufacturer. Quantitative real-time PCR assays were performed in triplicate to evaluate the effects of dietary SIM supplementation on the mRNA transcript abundance of Insulin-like Growth Factor-1 (IGF-1) and Lysozyme (LZ) in the liver of *L. calcarifer* juveniles. Real-time quantitative RT-PCR was performed using a real-time PCR machine (Rotor Gene-3000, Sydney, Australia) in a total volume of 12.5 μL containing 6.25 μL of SYBR Green qPCR Master Mix (×2) (Cinnagen, Iran), 0.5 μL of cDNA, 0.5 μL of each primer (Table 2), 0.1 μL Tag polymerase and 4.65 μL of double-

Table 2. Primers sequences and amplification efficiencies.

Gene name	Sequences of primers	Accession number	Efficiency	Product size
IGF-1	F: ACGCTGCAGTTTGTATGTGG R: CCTTAGTCTTGGGAGGTGCA	XM_018697285.1	98%	157
Lysozyme	F: GGTGTTTCTGCTCTTGGTGG R: GCCGTAGTCAGTGGATCCAT	XM_018667849.1	99%	196

Abbreviations: IGF-1, insulin-like growth factor I; LZ, lysozyme

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distilled and DNase free water. The Real-time quantitative RT-PCR program comprised denaturation step at 94°C for 2 min, followed by 40 amplification cycles of 15 seconds denaturation at 94°C, 30 seconds annealing at 60°C, 30 seconds extension at 72°C and a final extension at 72°C for 5 minutes. After PCR amplification, the melt-curve analysis was conducted to confirm that there was only one amplified product. The real-time PCR data analysis was performed in triplicate with Rotor-Gene, RG-3000 (Australia) software. The comparative method C_T ($2^{-\Delta\Delta CT}$) was used (Livak and Schmittgen, 2001).

Statistical Analysis

All data were analyzed using SPSS 16.0 (SPSS Inc., USA). The assumption of compliance of the data with the normal distribution was verified using the Kolmogorov–Smirnov test, while the assumption of homogeneity of variances was checked using the Levene tests. Differences between the dietary groups were tested using a one-way Analysis Of Variance (ANOVA) followed by Tukey's multiple comparison test. The significance level of each test was equal to 0.05. Data are presented as means±standard error (n= 3).

RESULTS

Physicochemical Factors of Water

The measurements of water quality parameters were as follows: temperature 30.12±2.5°C, oxygen 6.24±0.32 mg L^{-1}, salinity 40±0.82 g L^{-1}, and pH 7.8±0.22.

Immunological Parameters

The immunological parameters of Asian sea bass fed the experimental diets are given in Table 3. Total immunoglobulin level was significantly higher in all experimental groups than the value noted in the case of the control group. Serum lysozyme activity in Asian sea bass fed the *G. pygmaea* diets did not significantly change in comparison to the activity detected in the control individuals. Serum alternative complement (ACH50) activity was the highest in fish from the control and GL3 groups; they were significantly higher than the levels noted in the case of other treatments, i.e. GL6 and GL9 groups.

Antioxidant-Related Parameters

The activities of glutathione S-transferase and glutathione reductase are shown in Table 4. There was no significant difference in glutathione S-transferase activity as well as glutathione reductase activity between experimental and the control groups. The highest activity of glutathione S-transferase was found in the control group. The lowest value was detected in fish of GL9 group. Similarly, glutathione reductase activity was the highest in the case of control individuals. The lowest value was noted in GL6 group.

Intestinal Histology

Light microscopy revealed that the intestine of Asian sea bass fry fed either control diet or *G. pygmaea* supplemented diet showed an intact epithelial barrier and a mucosal arrangement of organized villi-like

Table 3. Immunological parameters of Asian sea bass fed diets with different levels of *G. pygmaea* for 42 days.

Parameters	C	GL 3	GL 6	GL 9
Total immunoglobulin (mg mL^{-1})	20 ± 0.52^{b}	23.83 ± 0.81^{a}	26.83 ± 0.31^{a}	23.9 ± 0.3^{a}
Lysozyme activity (U mL^{-1})	33.66 ± 0.88^{ab}	23.33 ± 0.88^{b}	36.67 ± 1.45^{a}	30.67 ± 2.96^{ab}
ACH50 (U mL^{-1})	135 ± 1.52^{a}	127 ± 4.04^{a}	$99 \pm1. 52^{b}$	107.33 ± 1.2^{b}

$^{a-b}$ Values in the same row not sharing a common superscript are significantly different (P< 0.05) SEM (Standard Error of the Means.

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Table 4. The antioxidant enzymes of Asian sea bass fed diets with different levels of *G. pygmaea* for 42 days.

Antioxidant enzyme measurements	C	GL3	GL6	GL9
Glutathione S-transferase (n mol mg^{-1} pr)	88.82±23.47 [a]	83.75±3.78 [a]	78.74±13.79 [a]	69.98±5.37 [a]
Glutathione reductase (n mol mg^{-1} pr)	7.47±1.34 [a]	5.09±1.07 [a]	3.66±0.04[a]	6.65±0.88 [a]

[a] Values in the same row not sharing a common superscript are significantly different (P < 0.05) SEM (Standard Error of the Means).

Table 5. Intestinal morphology of Asian sea bass fed the diets with different levels of *G. pygmaea*, for 42 days.

Parameters	C	GL3	GL6	GL9
Enterocyte height (µm)	12.48±0.97[a]	13.28±1.03[a]	14.58±0.85[a]	13.12±0.69[a]
Villi height (µm)	184.72±21.91[b]	222.22±40.26[a]	399.56±2.53[a]	211.26±23.12[a]
Muscularis thickness (µm)	19.88±1.37[a]	20.63±2.72[a]	23.01±0.22[a]	19.82±1.31[a]
Villi width (µm)	28.6±1.32[a]	31.5±3.73[a]	30.1±1.45[a]	26.56±1.12[a]

Values in the same row not sharing a common superscript are significantly different (P< 0.05) SEM (Standard Error of the Means).

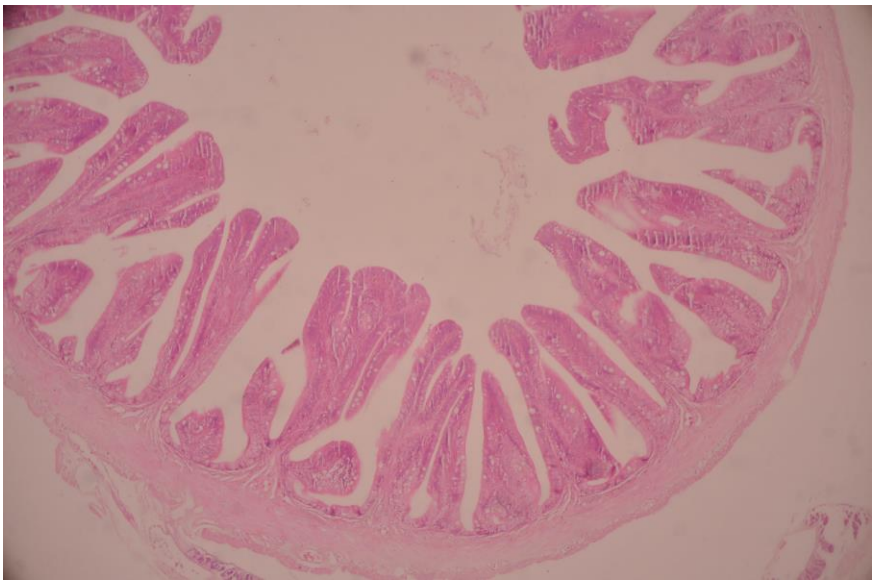

Figure 1. Transverse section of intestinal tissue of fish treated with 3% substitution (Magnification X 40).

mucosal folds (Figure 1). Histomorphometric data are shown in Table 5. Dietary supplementation of *G. pygmaea* had no significant influence on enterocyte height, villi width, and muscularis thickness in both intestinal regions. Nevertheless, the villi height in the anterior intestine was visibly lower in the control group as compared to other groups – changes were statistically insignificant.

Gene Expression

The amount of lysozyme mRNA transcript abundance in the liver of fish from the control group was visibly higher than the values detected in other groups – changes were statistically insignificant (Table 6).

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Table 6. Relative expression of IGF-I and lysozyme in the liver of Asian sea bass fed the diets with different levels of *G. pygmaea* for 42 days.

Parameters	C	GL3	GL6	GL9
Lysozyme	1.42±0.22[a]	1.01±0.65[a]	1.08±0.34[a]	1.11± 0.19[a]
IGF-I	5.28± 0.82[b]	1.02±0.15[a]	1.6± 0.26[a]	4.23± 0.73[b]

[a-b] Values in the same row not sharing a common superscript are significantly different (P< 0.05) SEM (Standard Error of the Means).

Fish from group GL9 and the control group exhibited significantly higher IGF-1 mRNA transcript abundance in the liver than groups GL3 and GL6.

DISCUSSION

In our experiment, total immunoglobulin level was higher in fish fed with the *G. pygmaea* diet in comparison to the control individuals. It may be a result of the presence of carotenoids in *Gracilaria* algae; carotenoids protect cells as antioxidants and prevent endangering cell health (Kumar *et al*., 2008). Similarly, it was reported that *Sargassum wightii* extract significantly increased serum globulin concentration in sutchi catfish (*Pangasianodon hypophthalmus*) (Prabu *et al*., 2016) and *Labeo rohita* (Gora *et al*., 2018). Zeynali *et al*. (2020) reported that total immunoglobulin level in the serum and skin mucus of Asian sea bass (*L. calcarifer*) dietary supplemented with *Sargassum ilicifolium* meal was increased, which suggest immunomodulatory effects. In contrast, Morshedi *et al*. (2018) demonstrated that serum total immunoglobulin level in Asian sea bass (*L. calcarifer*) gradually decreased with increasing dietary *G. pulvinata* levels, which suggests immunosuppressing effects at high inclusion levels. These findings indicated that seaweeds could modulate adaptive immune responses in fish.

In the present study, the replacement of *G. pygmaea* in the diet for Asian sea bass had no clear beneficial effect on lysozyme activity. However, Araujo *et al*. (2016) reported that rainbow trout (*Oncorhynchus mykiss*) fed a diet supplemented with 5% *Gracilaria* showed a significant increase in the lysozyme activity. Moreover, the study conducted by Morshedi *et al*. (2018) demonstrated that increased lysozyme activity was also observed in Asian sea bass (*L. calcarifer*) fed a diet supplemented with *Gracilaria* (3%). On the other hand, the results obtained by Valente *et al*. (2016) revealed that different levels of *Ulva* spp. meal added to the diet of Nile tilapia (*Oreochromis niloticus*) had no beneficial effect on lysozyme or peroxidase activities. Moreover, Van Doan (2014) reported that supplementation of the diet with Low Molecular Weight Agar (LMWA) did not affect lysozyme activity in basa (*P. bocourti*). Several factors such as species and size of fish, differences in diet formulation, and environmental conditions may result in the discrepancies observed among the aforementioned studies (Saurabh *et al*. 2008).

The mentioned findings of the experiments conducted by various authors indicate that seaweeds can modulate adaptive immune responses in fish. In the current experiment, fish fed with the *G. pygmaea* diet showed a reduced ACH50 activity. These results are similar to the results previously obtained by Morshedi *et al*. (2018) who reported that Asian sea bass fed with *Gracilaria*-supplemented diet showed a significant decrease in the complement system. Similar findings can be found in the research conducted by Araujo *et al*. (2016); the authors reported a decrease in ACH50 activity in rainbow trout (*O. mykiss*) fed with 10% *Gracilaria* spp. supplemented diet. The reason may rely on the antioxidant import carried by seaweed inclusion

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(Peixoto *et al*., 2016). However, the reason for the observed phenomenon is difficult to explain and further research is required.

In the current study, fish from all treatments had a similar appearance of the intact intestinal mucosal epithelium, well organized microvilli, and no cell debris in the lumen. The overall histological appearance of Asian sea bass sampled from all groups was normal. The morphometric parameters of intestine did not differ significantly. On the other hand, Araujo *et al*. (2016) reported a significant reduction in intestine diameter in fish fed with feed supplemented with 100 g kg^{-1} *Gracilaria vermiculophylla*. Moreover, seaweeds and seaweed-derived extracts affected intestinal morphology and resulted in changes in the digestion and absorption of nutrients in rainbow trout (*O. mykiss*) (Heidarieh *et al*., 2012) and Nile tilapia (*O. niloticus*) (Merrifield *et al*., 2011).

The shorter villi in GL3% group might be attributed to the presence of several ANFs in *Gracilaria* species, as proposed by Silva et al. (2015) which are resistant to proteolytic hydrolysis by digestive enzymes and harmful to the gut (Bardocz et al. 1995. Reactive Oxygen Species (ROS), which include hydroxyl radical, superoxide anion, hydrogen peroxide, and singlet oxygen, are physiologically generated in a series of biochemical reactions with in cellular compartments and increase in physiological conditions that result in oxidative stress, disease, and immune defense reactions (Dirks *et al*., 1982). The increased levels of ROS may lead to irreversible cell damage and eventually to cell death. Superoxide dismutase plays a crucial role in the defense against oxidative cell damage by catalyzing the breakdown of superoxide anion to oxygen and hydrogen peroxide (McCord and Fridovich, 1998). In normal cells, there exists a delicate balance between the pro-oxidant forces and antioxidant defenses known as "redox balance". Some researchers have previously reported that the overwhelming of antioxidant defenses of cells by pro-oxidants leads to oxidative stress. The oxidative stress is more profound in aquatic organisms than in others during nutritional deficiency, elevated temperature, hypoxia, and exposure to xenobiotics (Kolkovski *et al*., 2000; Romeo *et al*., 2000; Avanzo *et al*., 2002; Hwang and Li, 2002). However, an increase in ROS production above the level that can be removed by antioxidant defenses, or a decrease in the capacity of the antioxidant defenses, could result in oxidative damage to key molecules, including DNA and proteins (Halliwell and Gutteridge, 1999). In the present study no changes in redox balance was observed.

In the current study, IGF-1 mRNA transcript abundance in the liver increased with increasing amount of *Gracilaria pygmaea* in a dose-dependent manner indicating modulatory effects of *G. pygmaea* on the growth performance of Asian sea bass. On the other hand, the value detected in all supplemented groups was lower than the control value. No beneficial effects of *G. pygmaea* on the amount of lysozyme mRNA transcript abundance in fish liver were observed in the current study. Choi *et al*. (2014) reported that a brown macroalgae (*Hizikia fusiformis*) significantly enhanced plasma IGF-I levels that coincided with an increase in Liver pro-Inflammatory cytokines (IL-2 and IL-6) levels in juvenile olive flounder (*Paralichthys olivaceus*). On the other hand, Morshedi *et al*. (2019) revealed that *Lactobacillus plantarum* supplementation did not lead to significant difference in *IL* gene expression in *Sparidentex hasta*. The lack of clear positive effect of the studied diet modification on the gene expression may be related to *Gracilaria pygmaea* dose. Other studies confirm the concentration-dependent effects of immune stimulation on the effect at the molecular level (Yuan *et al*., 2008; Awad *et al*., 2011).

In conclusion, the results of the present study showed that *G. pygmaea* meal exhibits some potential as a fishmeal replacement for inclusion in diets for Asian sea bass. Dietary inclusion can be used without detrimental effects on immunity, antioxidant status,

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digestive system morphology, and *IL-1β* and *IGF-1* gene expression. No considerable toxicity of *G. pygmaea* was detected in the present study.

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اثرات جایگزینی نسبی ماکرو-جلبک جیره (Gracilaria pygmaea) بر پاسخ ایمنی، بافت روده، فعالیت آنزیم¬های آنتی اکسیدانی و بیان ژن ماهی سي باس آسیایي(*Lates calcarifer*)

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چکیده

ماهی باس آسیایی (Lates calcarifer) یک گونه دریایی مهم با ارزش اقتصادی بالا و کیفیت گوشت عالی است که به دلیل بیماری در آبزی پروری با تراکم بالا متحمل تلفات زیادی شده است. جلبک ماکرو، Gracilaria pygmaea، که شامل ترکیبات فعال زیستی مختلف است، ممکن است به عنوان یک محرک ایمنی در صنعت آبزی پروری عمل کند. هدف از این تحقیق بررسی اثر کاربرد آرد جلبک گراسیلاریا Gracilaria pygmaeaبه صورت جایگزینی در جیره غذایی و تاثیر بر ایمنی، بافت روده، آنزیم¬های آنتی اکسیدانی و بیان ژن ماهی سی باس آسیایی Lates calcarifer بود. بدین منظور ۱۲۰ قطعه ماهی سی باس آسیایی با میانگین وزنی ۰/۵±۲۸ گرم به چهار تیمار و سه تکرار در ۱۲ تانک فایبرگلاس ۳۰۰ لیتری تقسیم شد. تیمار¬های آزمایش بر اساس چهار جیره غذایی هریک به ترتیب حاوی ۰، ۳، ٦ و ۹ درصد آرد جلبک گراسیلاریا تهیه گردید. در پایان آزمایش از سیاهرگ ساقه¬ی دمی ماهیان خونگیری انجام و پلاسمای خون جهت اندازه¬گیری پارامتر¬های ایمنی و برش عرضی روده جهت بافت شناسی و کبد جهت آزمایش آنزیم

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آنتی اکسیدانی و بیان ژن به آزمایشگاه منتقل گردیدند. نتایج این تحقیق نشان داد که افزودن جلبک گراسیلاریا در جیره غذایی ماهی سی باس آسیایی در سطح ۳% جایگزینی آرد جلبک گراسیلاریا باعث افزایش در میزان آنزیم کاتالاز می¬گردد. همچنین بالاترین سطح ایمنوگلوبولین در تیمار ٦% آرد جلبک گراسیلاریا مشاهده گردید. نتایج حاصل از بررسی بافت روده در تیمار¬های مورد مطالعه نشان داد که ساختار ویلی¬ها و انتروسیت¬های دستگاه گوارش در تیمار¬هایی که از آرد جلبک استفاده کرده¬اند کاملاً طبیعی بوده و هیچ گونه ضایعات بافت شناسی مشاهده نشد. ساختار لایه¬های مخاطی، عضلانی و سروز و همچنین سلول¬های موکوس روده¬ای عادی و تغییرات چندانی را نشان نداد. نتایج حاصل از بیان ژن بیانگر افزایش سطح فاکتور رشد شبه انسولینی در سطح ۹% و سطح کنترل شده است.

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