



DIETARY ADMINISTRATION OF GREEN MACROALGAE (*ULVA INTESTINALIS*) ON GROWTH PERFORMANCE, SERUM IMMUNE PARAMETERS, AND GENE EXPRESSION IN COMMON CARP (*CYPRINUS CARPIO*)

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Abstract

The purpose of this experiment was to assess the effects of the green macroalgae (*Ulva intestinalis*) on growth performance, immune parameters of serum and mucus, liver enzymes and the expression of genes related to growth (GH and IGF-1), and immunity (IL1- β and LYZ) in common carp (*Cyprinus carpio*). The test was carried out in eight weeks with the treatments supplemented with 0 (control), 0.25%, 0.5%, and 1% macroalgae and three replications for each group. At the end of the course, growth indices, serum and mucus total protein, total immunoglobulin, lysozyme, and liver enzymes including alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were evaluated. Additionally, the expression of growth (GH and IGF-1) and immunity (IL1- β and LYZ) genes was evaluated using real-time PCR in the brain, liver, and intestine. Based on the results, a significant difference was observed in the levels of total protein, total immunoglobulin, and mucus lysozyme in the fish that received 1% *U. intestinalis* compared to the control group ($P < 0.05$). In addition, the amount of total protein, total immunoglobulin, and lysozyme in the control group and the other treatments represented a significant difference ($P < 0.05$). Although the initial weight did not show a significant difference among the treatments ($P > 0.05$), the final growth parameters significantly varied in different treatments, with the highest final weight observed in fish fed with 0.25% algae ($P < 0.05$). The results of the expression of immune genes lysozyme (LYZ) and interleukin-1 (IL-1) indicated a significant difference with the control group ($P < 0.05$), representing an increasing pattern with the rising percentage of macroalgae ($P < 0.05$). The supplement was also found effective in promoting the expression of growth hormone (GH) and insulin-like growth factor (IGF-1) compared to the control fish ($P < 0.05$), revealing an increasing pattern for the IGF-1 gene with the elevated level of the supplement. In general, according to the results of the present study, *U. intestinalis* can be recommended in carp aquaculture, most preferably at the level of 1% of daily feed, as it could significantly enhance the growth and innate immunity of fish.

Key words: *Ulva intestinalis*, immunity, growth, genes expression, common carp

The aquaculture industry, a vital source of human food supply, faces diverse challenges, including the optimization of fish growth and health through diet supplementation (Niroomand et al., 2011; Lieke et al., 2020). Carp, renowned for its adaptability and nutritional value, plays a pivotal role in addressing these challenges (Zhou et al., 2020). Its widespread cultivation not only sustains global food demands but also contributes to the industry's growth by enhancing diet strategies for fish health and growth optimization. The use of additives is a recommended method to improve growth indicators, survival rates, and food efficiency in farmed fish (Guerreiro et al., 2019; Pezeshk et al., 2019; Doan et al., 2020; Hoseinifar et al., 2020; Marques et al., 2020; Klongklaew et al., 2021; Safari et al., 2021). In recent years, the use

of microbial food supplements (probiotics, prebiotics and synbiotics), organic acids and their salts, hormones, algae (micro and macroalgae), and plant extracts in the diet to elevate the growth of aquatic animals has been considered (Srikong et al., 2017; Safavi et al., 2019; Klongklaew et al., 2021; Pradhan et al., 2021; Ruslan et al., 2021). Algae are considered a rich source of useful and biologically active compounds (Garcia-Poza et al., 2020; Ruslan et al., 2021). The cell wall of algae contains secondary compounds and valuable polysaccharides such as carrageenan, agar, and alginic acid, which are widely used in medicine and industry (Dawes et al., 1998). In addition, many biological compounds with a wide range of applications, including antibiotic, antiviral, and antifungal effects, have been identified and

derived from multicellular algae. The positive effects of algae and substances extracted from them on the growth and immunity of fish have been reported in different studies, such as red algae (*Porphyra purpurea*) in gray mullet (*Chelon labrosus*) (Davies et al., 1997), *Gracilaria cornea* in European sea bream (*Dicentrarchus labrax*) (Valente et al., 2006), *Sargassum angustifolium* in common carp (*Cyprinus carpio*) (Karami et al., 2016), *Sargassum bovinum*, *Polycladia myrica* and *Gracilariopsis persica* in rainbow trout (*Oncorhynchus mykiss*) (Kazemi et al., 2018), *Gracilaria pygmaea* and *Sargassum cristae-folium* in rainbow trout (Sotoudeh, 2020), *Enteromorpha intestinalis* in Nile tilapia (*Oreochromis niloticus*) (Siddik et al., 2015), *Ulva* family algae in spotted scat fish (*Scatophagus argus*) (Yangthong et al., 2020), *Enteromorpha prolifera* algae in crucian carp (*Carassius auratus*) (Zhou et al., 2020), *Ulva lactuca* and *Jania rubens* mixture in red swamp crayfish (*Procambarus clarkii*) (Mazlum et al., 2021) and *Ulva ohnoi* algae in sole fish (*Solea senegalensis*) (Ponce et al., 2020).

U. intestinalis (syn. *Enteromorpha intestinalis*), which is placed in Chlorophyceae class, Ulvales order, Ulvaceae family, and *Enteromorpha* genus is characterized by green filaments, flat fibers, and usually folded edges. In addition to chlorophyll a and b, this alga also contains alpha- and beta-carotene, lutein, and xanthine. In terms of distribution, it lives in the upper tidal area of warm sea waters and is sometimes found in the brackish waters of estuaries and even freshwater ponds (Sohrabipour and Rabiei, 2007).

Antimicrobial (Valente et al., 2006, 2016; Ravi Kumar et al., 2009; Taskin et al., 2007; Khezri Ahmad Abad et al., 2016; Daneshvar Ghorbani et al., 2019), and antioxidant properties (Hashem Dabbagian et al., 2015) of this alga have been reported in previous studies. This alga is also known for its high protein content, making it a suitable choice to improve the structure and nutritional value of animal and human food (Pirian et al., 2017).

Because so far, a study on the effects of utilizing green macroalgae (*U. intestinalis*) on the immune function of serum and mucus, growth parameters, liver enzymes and the expression of genes related to growth, immunity has not been done in common carp (*C. carpio*), the present study was based on the aim of investigating the mentioned parameters.

Material and methods

Treatments and food preparation

Fish were prepared from a private farm located in Zarrin Shahr (Isfahan, Iran). Water was supplied from a well as flow through and distributed in twelve 100 L aerated aquariums each containing 25 fish in four groups each in three replicates. An overall number of 300 carp with an average weight of 22 ± 1 g were adapted for two weeks to the test conditions. The proximate composition of the commercial basal feed used in the study are shown

in Table 1. The fish received diets containing 0, 0.25%, 0.5%, and 1% of the macroalgae. The feeding rate was adjusted at 3% of body weight, three times daily during the 8 weeks of the experiment.

Table 1. Composition of the basal diet used throughout the experiment

Proximate analysis (%)	
Dry matter	93.6
Crude protein	38.9
Crude lipid	15.0
Ash	11

Growth parameters

At the end of the period, all the fish in each tank were weighed separately using a digital scale, and the weight gain, specific growth rate, and food conversion ratio were calculated using the following formulas as indicators of growth performance.

Weight gain (WG, g) = FW – IW

Feed conversion ratio (FCR) = feed intake (g) / (FW – IW)

Specific growth rate (SGR, %/d) = $100 \times [(\ln \text{FW} - \ln \text{IW}) / \text{days}]$

Survival rate (SR, %) = $100 \times (\text{final number} / \text{initial number})$

FW: final weight; IW: initial weight.

Measurement of serum biochemical parameters and immunity

Measurement of serum biochemical parameters

Biochemical and innate immune parameters were measured in the fish serum at the end of the course. After anesthesia with clove oil, blood samples were taken from three fish in each of the replicates, and the serum was collected according to Safari et al. (2017). Biochemical parameters, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes, were measured using a commercial kit (Pars Azmun, Co., Tehran, Iran) and an auto-analyzer (Prestige 24i, Boeki, Japan) with the IFCC enzyme method according to the instructions by Reitman and Frankel (1957). Alkaline phosphatase (ALP) and total blood serum protein were measured photometrically using commercial kits (Pars Azmun, Co., Tehran, Iran), according to the manufacturer's instructions (Fishbach and Zawta, 1992).

Serum innate immunity parameters

1. Determination of lysozyme activity

Plasma lysozyme activity was measured according to the method of Clerton et al. (2001). The method is based on the lysing activity of the enzyme on the lysozyme-sensitive Gram-positive bacteria (*Micrococcus luteus*). Briefly, 0.2 mg mL⁻¹ of the bacterium (Sigma, USA) was dissolved in phosphate buffer (0.05 M), to create a suspension. Then 100 microliters of plasma were mixed

with 2 milliliters of the suspension in 96-well microplates. The optical absorbance of the samples was read every 30 seconds for 5 minutes at 25°C using an ELISA reader (BioTek, Winooski, USA) at a wavelength of 530 nm. Egg albumen (Sigma, St. Louis, USA) was used to prepare a standard curve, and each unit of lysozyme activity was determined based on the decrease in absorbance (0.001/min).

2. Determination of total immunoglobulin level

Total immunoglobulin was calculated according to the protein content before and post addition of polyethylene glycol (Siwicki and Anderson, 1993).

3. Measurement of mucus immune parameters

The sampling of mucus was carried out according to the method previously described by Subramanian et al. (2007). Briefly, three fish were caught from each replication and after anesthetization were transferred to a tank containing clean water to minimize the external bacteria. The fish were immediately placed into zip-plastic bags containing 5 ml sodium chloride, 50 ml molar and were gently rubbed to collect mucus. After a period of two minutes, the fish were transferred to tanks with proper oxygenation. Mucus/serum was collected from the bags and its supernatant was obtained in the laboratory after centrifugation (1500 rpm for 10 minutes at 4°C) and was kept at -80°C before further analysis (Siwicki and Anderson, 1993).

Expression of growth and immunity genes

Sampling

At the end of the period, samples were taken from 9 fish in each treatment to evaluate the IGF-1 (liver), GH (brain), IL1- β , and LYZ (intestine). The procedure was done under sterile conditions, with the samples being transferred into cryotubes in liquid nitrogen, before being stored at -80°C for further processing.

RNA extraction and gene expression assays

The RNA was extracted from 100 mg of tissue samples of the liver, intestines, and brain homogenized with liquid nitrogen, using the Esterabad-ZistFan-Pishro-Azma kit, according to the manufacturer's instructions. The quality of total RNA was determined using electrophoresis on 1.5% agarose gel and the absence of DNA contamination was authenticated using a nanophotometer (IMPLEN-P100) at the wavelength of 260 to 280 nm. The production of the cDNA was carried out according to Genet Bio (South Korea). The synthesized cDNAs were kept at -20°C before the experiments. The qPCR reactions were conducted in a final volume of 20 microliters by Amplicon's cyber kit in the company's iQ5 device (Bio-Rad, USA). Beta-actin gene was selected as the reference primer (Safari et al., 2017). The samples of brain, liver, and intestine tissues were tested in 4 technical repetitions at optimal primer temperature. In order to ensure the optimality of qPCR conditions, a series of different

concentrations of mixed cDNA samples from different tissues relating to different treatments were prepared and were multiplied with both target and reference primers in three repetitions. Then a standard curve was drawn to estimate the efficiency (E) and repeatability of the test for each primer (Bustin et al., 2009).

Statistical analysis

The normality of data and homogeneity of variances was tested using the Kolmogorov-Smirnov and Levene's tests. The obtained data were analyzed using one-way analysis of variance at a confidence level of 95% and the comparison between means was carried out with Duncan's test using SPSS software (Ver. 19, Chicago, IL, USA). Graphs were drawn using Microsoft Excel (Microsoft, 2010).

Results

Growth parameters

The results of the growth parameters are presented in Table 2. While there was no significant difference between the initial weights of fish distributed in different treatments ($P>0.05$), growth-related parameters significantly changed at the end of the trial. The fish supplemented with the macroalgae showed significantly refined growth parameters compared to the control ($P<0.05$), with the highest means in those fed with 0.25% algae. Similarly, the supplemented fish represented a higher percentage of survival when compared to the control.

Innate immunity parameters of serum and mucus

The results of the immune parameters of blood serum and mucus of common carp fed with *U. intestinalis* are given in Table 3. Based on the results, the highest levels of serum total protein, total immunoglobulin, and lysozyme were found in the fish receiving 1% macroalgae (0.95 ± 0.12 , 0.57 ± 0.11 , and 9.4 ± 0.7), indicating a significant difference with the control ($P<0.05$). However, these parameters did not represent a statistical difference between the fish supplemented with lower amounts of *U. intestinalis* (0.25% and 0.5%) and those of the control group ($P>0.05$).

Significant differences were found in total protein, total immunoglobulin, and lysozyme levels between supplemented and unsupplemented fish ($P<0.05$). Similar to mucus, the highest values of serum total protein and lysozyme (1.98 ± 0.22 and 22.8 ± 0.31) were observed in 1% algae treatment. The highest levels of total serum immunoglobulin were observed in 1% and 0.5% treatments, respectively (1.14 ± 0.17 and 1.26 ± 0.11).

Liver enzymes

The results for liver enzymes, shown in Figures 1–3, represented no significant differences among treatments in terms of AST, ALP and ALT activities.

Table 2. Growth performance of *C. carpio* fed with different green macroalgae *U. intestinalis* during 8 weeks (n=9; mean $\pm$ SD)

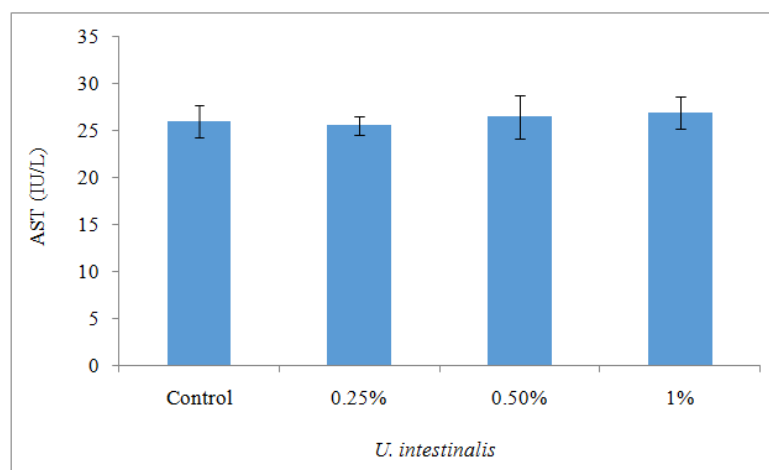
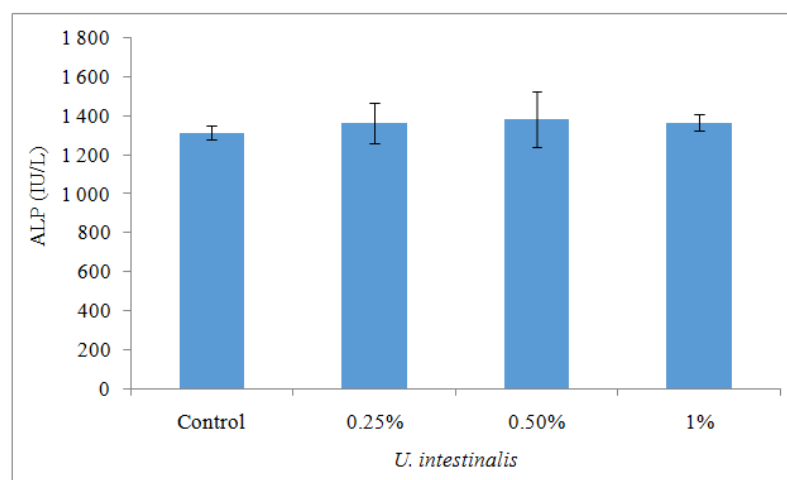
Parameters	Control	0.25%	0.05%	1%
IW (g)	23.24 $\pm$ 0.06	23.29 $\pm$ 0.16	23.23 $\pm$ 0.22	23.39 $\pm$ 0.04
FW (g)	27.20 $\pm$ 0.29 c	30.28 $\pm$ 0.16 a	29.02 $\pm$ 0.13 b	29.13 $\pm$ 0.11 b
WG (g)	3.95 $\pm$ 0.22 c	6.98 $\pm$ 0.01 a	5.78 $\pm$ 0.35 b	5.74 $\pm$ 0.15 b
SGR (% d ⁻¹)	0.37 $\pm$ 0.01 c	0.62 $\pm$ 0.01 a	0.52 $\pm$ 0.03 b	0.52 $\pm$ 0.01 b
FCR	2.22 $\pm$ 0.24 a	1.62 $\pm$ 0.01 b	1.97 $\pm$ 0.12 b	1.97 $\pm$ 0.05 b
SR (%)	88.88 $\pm$ 2.22 b	100.0 $\pm$ 0.00 a	100.00 $\pm$ 0.00 a	100.00 $\pm$ 0.00 a

Different letters characterize significant difference (P<0.05). IW: initial weight; FW: final weight; WG: weight gain; SGR: specific growth rate; FCR: feed conversion rate; SR: survival rate.

Table 3. Immune performance of serum and mucus of *C. carpio* fed with different green macroalgae *U. intestinalis* during 8 weeks (n=9; mean $\pm$ SD)

Parameters	Control	0.25%	0.5%	1%
Mucus total protein (g/dl)	0.75 $\pm$ 0.05 b	0.81 $\pm$ 0.05 b	0.87 $\pm$ 0.03 ab	0.95 $\pm$ 0.12 a
Mucus total immunoglobulin (g/dl)	0.42 $\pm$ 0.07 b	0.49 $\pm$ 0.011 ab	0.54 $\pm$ 0.02 ab	0.57 $\pm$ 0.11 a
Mucus lysozyme (U/ml)	7.53 $\pm$ 0.075 b	7.96 $\pm$ 0.68 b	8.43 $\pm$ 0.6 ab	9.4 $\pm$ 0.7 a
Serum total protein (g/dl)	1.64 $\pm$ 0.086 c	1.82 $\pm$ 0.25 b	1.8 $\pm$ 0.17 b	1.98 $\pm$ 0.22 a
Serum total immunoglobulin (g/dl)	1.03 $\pm$ 0.2 b	1.02 $\pm$ 0.06 b	1.26 $\pm$ 0.11 a	1.14 $\pm$ 0.17 a
Serum lysozyme (U/ml)	20.23 $\pm$ 0.14 c	21.57 $\pm$ 0.39 b	23.1 $\pm$ 0.43 a	22.8 $\pm$ 0.31 a

Different letters characterize significant difference (P<0.05).

Figure 1. Measurement of AST activity of *C. carpio* fed with different green macroalgae *U. intestinalis* during 8 weeks (n=9; mean $\pm$ SD)Figure 2. Measurement of ALP activity of *C. carpio* fed with different green macroalgae *U. intestinalis* during 8 weeks (n=9; mean $\pm$ SD)

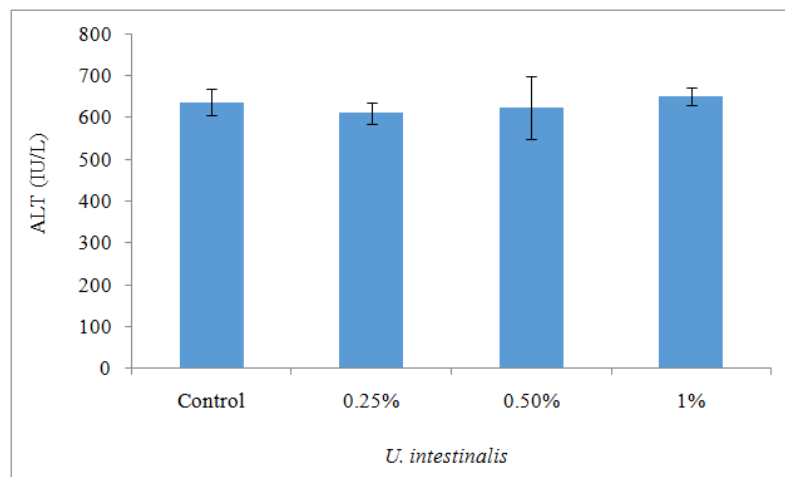


Figure 3. Measurement of ALT activity of *C. carpio* fed with different green macroalgae *U. intestinalis* during 8 weeks (n=9; mean ± SD)

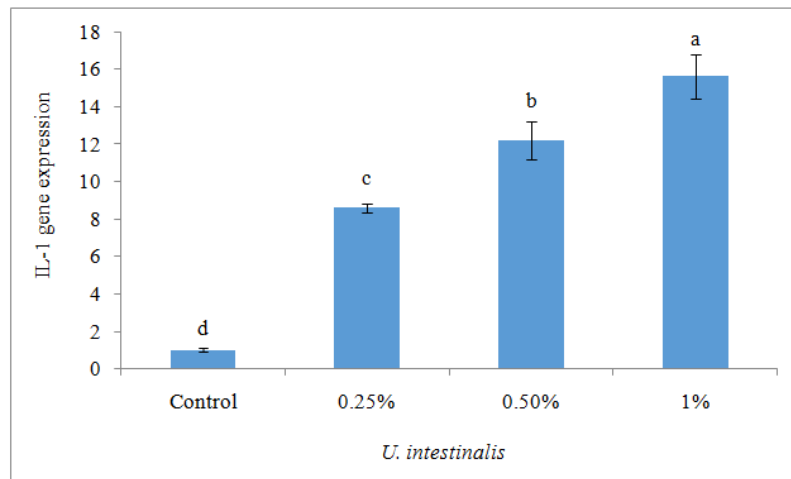


Figure 4. Alternative in relative IL-1 gene expression of *C. carpio* fed with different green macroalgae *U. intestinalis* during 8 weeks (n=9; mean ± SD). Different letters characterize significant difference (P<0.05)

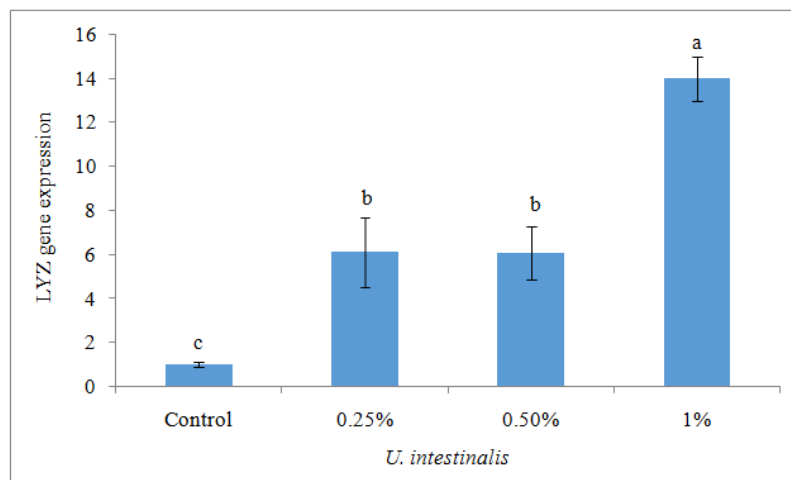


Figure 5. Alternative in relative LYZ gene expression of *C. carpio* fed with different green macroalgae *U. intestinalis* during 8 weeks (n=9; mean ± SD). Different letters characterize significant difference (P<0.05)

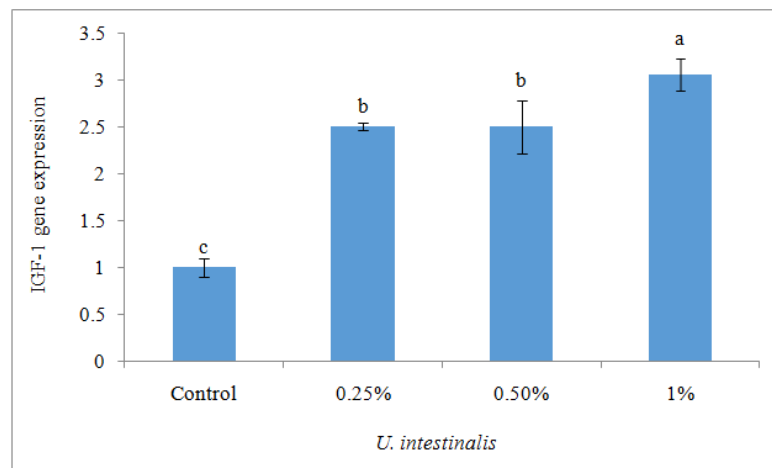


Figure 6. Alternative in relative IGF-1 gene expression of *C. carpio* fed with different green macroalgae *U. intestinalis* during 8 weeks (n=9; mean ± SD). Different letters characterize significant difference ($P<0.05$)

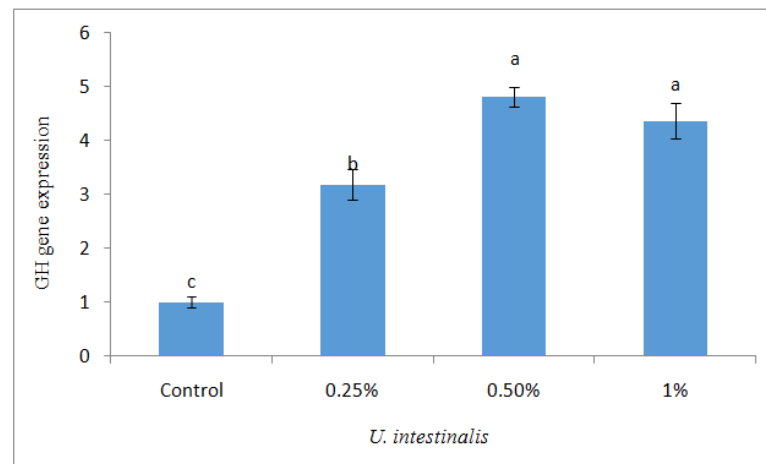


Figure 7. Alternative in relative GH gene expression of *C. carpio* fed with different green macroalgae *U. intestinalis* during 8 weeks (n=9; mean ± SD). Different letters characterize significant difference ($P<0.05$)

The results of gene expression in common carp

A significant difference was found in the expression of interleukin 1 (IL-1) and lysozyme (LYZ) genes in supplemented fish with the control group ($P<0.05$), which was dose-dependent for the gene coding IL-1 ($P<0.05$). Similarly, the expression of LYZ gene significantly improved in the fish fed with macroalgae compared to the control group. The fish that received 1% algae showed the highest level of LYZ gene expression, followed by those receiving 0.5% and 0.25% ($P<0.05$; Figures 4 and 5).

The results also indicated a significant difference in the expression of growth-related genes (growth hormone (GH) and insulin-like growth factor IGF-1) ($P<0.05$). Notably, akin to LYZ gene, the upsurge in IGF-1 gene expression exhibited an increasing pattern with the macroalgae dosage increasing, representing a significant difference between treatments and control fish ($P<0.05$).

GH-coding gene was also found significantly up-regulated in the supplemented fish than the control group ($P<0.05$) with the highest expression in 1%- and 0.5%-supplemented treatments (Figures 6 and 7).

Discussion

Macroalgae and their extracts hold tremendous promise for the aquaculture sector, garnering considerable attention in recent years due to their capacity to bolster the health and productivity of aquatic animals, thereby providing consumers with a healthy food source (Vijayaram et al., 2024). Although green macroalgae constitute less than 1% of the total macroalgal biomass, they are among the most extensively researched macroalgae in aquaculture due to their superior properties. Moreover, studies indicate that macroalgae and derived products can

positively impact parameters of the innate immune response and antioxidant defense in aquatic organisms. In our study, carp fed with diets supplemented with *U. intestinalis*, a common species of green macroalgae exhibited increased growth and improved immunity. These findings are further supported by molecular investigations.

Growth

In the current study, the supplementation of green macroalgae demonstrated a positive impact on the growth performance of common carp, particularly with the most substantial increase observed in the group supplemented with 0.25%. In parallel with our study, Akbary and Aminikhoei (2018 a) documented that the inclusion of 1.5 g/kg polysaccharides derived from the green macroalga *U. rigida* enhanced growth in shrimp *Litopenaeus vannamei*. In a subsequent study conducted utilizing the same macroalga involving grey mullet (*Mugil cephalus*), the authors reported that the optimal growth enhancement was achieved with a supplementation of 1 g/kg (Akbary and Aminikhoei, 2018 b).

The results of the study by Wassef et al. (2005) on two macroalgae, *Ulva lactuca* and *Pterocladia capillacea*, represented that utilization of 5% *U. lactuca* and *P. capillacea* in the diet of sea bass (*D. labrax*) improved growth rate. Similarly, the results of Mustafa (1995) on *Pagrus major* showed that feeding with three types of macroalgae, *Ascophyllum nodosum*, *Porphyra yezoensis*, and *Ulva pertusa* improved growth by 0.5%. The study of Soler-Vila (2009) also indicated that red macroalgae *Porphyra dioica* had a positive effect on the growth of rainbow trout up to the level of 15% of the diet. Several studies have been conducted on the importance of macroscopic algae as protein sources in the diet of aquatic animals and its effect on growth performance, carcass composition and digestive enzyme activity in different aquatic species (Akbary and Aminikhoei, 2018 a; Mazlum et al., 2021). The impact of macroalgae on growth performance could stem from their richness in protein, essential amino acids, and vitamins (Becker, 2004). Farhoudi et al. (2017) similarly reported that the replacement of different levels of *Gracilaria* red algae in the Asian seabass (*Lates calcarifer*) diet resulted in a higher final weight in the fish fed with 6% *Gracilaria*.

Similar to the present study, they investigated the effects of food supplementation with red seaweed (*G. pygmaea*) on the growth, carcass composition and hematology of rainbow trout (*O. mykiss*), resulting in the final weight increase in the fish that were fed 6% algae, significantly higher than control fish and those that received 12% algae (Sotoudeh and Mardani, 2017).

Fish growth is regulated by factors such as IGF-1, IGF-2, and myostatin, which play pivotal roles in myogenesis (Zhong et al., 2022). Research has demonstrated that the expression of IGF-1 and IGF-2 is influenced by diet (Gabillard et al., 2006). The action of growth hormone (GH) encompasses both direct and indirect effects

on the body. Direct effects involve an increase in protein synthesis, including RNA synthesis and amino acid absorption, while indirect effects occur through the release of two types of IGF, namely IGF-1 and IGF-2 (Humbel, 1990). Numerous studies have already established a positive correlation between blood IGF-1 or tissue mRNA levels and dietary content, protein levels, and overall body growth rate across various fish species (Reinecke et al., 2005).

The results of the expression of growth hormone (GH) and insulin-like growth factor (IGF1) genes represented a significant improvement compared to the control group, with the highest level in the common carp supplemented with 1% *U. intestinalis* ($P < 0.05$). In accordance with our study, the supplementation of macroalgae has been observed significantly effective in the upregulation of growth-related genes, including GH and IGH1, in zebrafish (Mahmoudi et al., 2022).

Although there are numerous findings approving the positive role of macroalgae in fish growth, some reports have represented inconsistent results. Yildirim et al. (2009) also showed that adding *Ulva* and *Enteromorpha* at a rate of 10% for 60 days to the diet of rainbow trout did not affect the specific growth rate (SGR) and relative growth percentage (RGR) compared to the control group. They found that although the ratio of protein efficiency (PER) and survival was higher in supplemented fish, they represented a lower growth rate than the control group (Yildirim et al., 2009).

Immune system

Particularly at low inclusion levels, modulating both the humoral and cellular components of the innate immune system is recognized as one of the observed immunostimulatory effects of macroalgae (Mahmoudi et al., 2022; Rouhani et al., 2022). The immune responses in fish fed with a diet containing macroalgae can be contradictory, and the effects of macroalgae can vary significantly. Therefore, it has been reported that testing the outcome is necessary for each fish species and diet formulation (Fumanal et al., 2020).

Total protein, total immunoglobulin, and lysozyme, which are significant indicators of the non-specific immune system, are commonly used parameters in monitoring changes in the non-specific immune system in fish. The results of the current study indicated that the levels of total protein, total immunoglobulin, and lysozyme were significantly higher than the control group. In line with the results of this study, Zamannejad et al. (2016) investigated the nutritional effect of brown sarcoma algae on changes in immunoglobulin (IgM) and lysozyme levels in rainbow trout (*O. mykiss*) and reported that immunoglobulin levels and activity of lysozyme increased with increasing the amount of *Sargassum* algae. Similarly, Rouhani et al. (2022) indicated that the supplementation of zebrafish (*Danio rerio*) with green macroalgae (*Ulva intestinalis*) could significantly promote systemic immune parameters, including protein and globulin lev-

els, and lysozyme activity. However, the concentration of immune indices in mucus was elevated only in the fish that received 1% of the algae.

Akbary and Aminikhoei (2019), consistent with our study, revealed a significant increase in lysozyme activity in mullet fish that were fed a diet supplemented with 1% water-soluble extract of *Ulva rigida* compared to those fed the control diet and other treatment levels.

Furthermore, the addition of 5% *U. ohnoi* to the diet of *S. senegalensis* has been reported to modulate the immune response during *Photobacterium damsela* subsp. *piscicida* infection and result in a reduction in pathogen numbers in the liver (Fumanal et al., 2020). In the study of Valente et al. (2016) on tilapia fish, serum lysozyme increased in the treatment fed the diet containing 5% *Ulva* macroalgae powder compared to the control treatment and those that received 10% *Ulva* macroalgae.

At the molecular level, there was also a significant difference in the expression of immune related genes (IL-1 and LYZ) in the fish supplemented with green macroalgae compared to the control group. In certain studies, involving various macroalgal species, similar positive effects on immune related genes have been demonstrated. Supplementing zebrafish with *Dictyota dichotoma* has also been reported as significantly effective in the expression of LYZ and IL-1 genes (Mahmoudi et al., 2022). Moreover, Hoseinifar et al. (2022) reported that the addition of 0.5% red algae led to an increase in the expression levels of interferon-gamma (IFN- γ), tumor necrosis factor-alpha (TNF- α), and interleukin-1 beta genes in zebrafish.

The effect of feeding with hydroalcoholic extract of red algae (*Laurencia caspica*) on antioxidant defense, immune responses, and kidney immune gene expression in rainbow trout (*O. mykiss*) infected with *A. hydrophila* was investigated, which resulted in the upregulation of immune genes (IL-1 β , lysozyme II, complement C3 and TNF α) after bacterial challenge.

Vazirzadeh et al. (2020) also reported that diets containing seaweed could significantly enhance the expression of LYZII, TNF α , and IL-1 β compared to control fish in rainbow trout. However, these stimulatory effects were best at lower levels and shorter feeding times. Nevertheless, Hoseinifar et al. (2018) documented that the supplementation of red macroalgae resulted in non-significant differences in the expression of TNF- α , LYZ, and IL-1 in zebrafish.

The variations in the immunostimulatory effects of seaweed may be attributed to factors such as the extraction method, testing conditions, fish species, and so forth. Phytochemical investigations have revealed that seaweed comprises a plethora of compounds including carbohydrates, alkaloids, steroids, phenols, saponins, carotenoids, and flavonoids (Mansuya et al., 2010), all of which play crucial roles in stimulating the immune system. Ghasemi Pirbalouti et al. (2011) attributed the increase in rainbow trout immune factors to the pres-

ence of various phytochemical compounds such as phenolic compounds, steroids, flavonoids and terpenoids existing in the essential oil of oregano, marzeh and plant resin.

Biochemical parameters

Previous studies have shown that application of dried algae as an immune stimulant in the aquaculture can improve physiological responses to stress and disease (Jaime-Ceballos, 2005).

The liver, serving as a vital organ, plays a crucial role in numerous metabolic processes of nutrients. Aminotransferases such as AST and ALT, essential for the Krebs cycle, are generally considered indicators of protein metabolism (Xuan et al., 2019). In this study, no changes were observed in the ALT, AST, and ALP activities in the liver of fish fed with *U. intestinalis* containing diets.

Elevated levels of ALT and AST in the serum are frequently recognized as markers of liver and heart damage, while heightened serum activities of ALP, AST, and ALT are generally interpreted as the organism's response to stressors (Jahanbakhshi et al., 2021). Nevertheless, the lack of alterations in ALT, AST, and ALP activities observed in this study might be attributed to the absence of stress induction by *Ulva* in carp.

Conclusion

Considering the results obtained from this study, it can be concluded that incorporating *U. intestinalis* into the diet of common carp can lead to improvement in the growth-related indicators, especially the final weight, weight gain, and weight gain percentage. Investigation of non-specific immune factors demonstrated that the inclusion of *U. intestinalis* increased lysozyme activity, total protein, and total immunoglobulin. Analysis of growth and expression of immunity-related genes in common carp represented a significant difference in the treatments fed with macroalgae than the control fish. Moreover, the study revealed that the inclusion of *Ulva* did not induce any stress in carp. Based on the findings of our research, *U. intestinalis* can be effectively incorporated at a level of 1% in aquaculture practices.

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Ethical approval

We adhered to the applicable guidelines for the care and utilization of animals, as outlined in the National Ethical Framework for Animal Research in Iran. The execution of all experiments followed the approved protocol established by the Ethics Committee of the Gorgan University of Agricultural Sciences and Natural Resources (GUASNR: 99-430-91).

Declaration of competing interest

The authors declare no competing interests.

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