Investigation of the effect of diet containing Red algae (Laurencia caspica) on blood parameters and activity of digestive enzymes of goldfish (Carassius auratus)

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Abstract

The present study was conducted to investigate the effect of the diet containing Laurencia caspica (red algae) on blood parameters and digestive enzymes activity of goldfish after 90-day period. Goldfish (weight 6.10±0.1 gr) were divided to four experimental groups (30 individuals in each replication) including experimental treatment of the control group (without using red algae) and three treatments by adding 5, 10, and 20 grams of red algae to kilogram of diet. The results of the experiment showed that all hematological indices (red blood cells, white blood cells, hemoglobin, and hematocrit) in fish fed with algae significantly increased compared to the control group ($P < 0.05$). Among the blood biochemical indices, there were a significant difference in the triglycerides and cholesterol ($P < 0.05$). The highest activities of the digestive enzymes were observed in the treatment containing 20 grams of algae per kilogram of diet ($P < 0.05$) including amylase (620.26± 4.10 U/mg protein), lipase (19 ± 1.17 U/mg protein), and protease (290.11 ± 3.20 U/mg protein). Overall, the results of the study showed that there was a significant improvement in blood indices and the activity of digestion enzymes with the use of a diet containing 20 grams of red algae per kilogram of goldfish diet and therefore this diet is recommended.

Keywords: red algae, hematological indices, biochemical parameters, digestive enzyme.

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Introduction

Over recent years, the research in the field of making use of sea algae has attracted attention throughout the world due to their medicinal properties and the studies on this subject provide valuable findings every year. The sea algae are converted into the natural materials widely used in the food and medical industries due to low levels of saturated fats and calories and containing plenty of carbohydrates, and their biological properties such as antibacterial properties, antioxidative properties, and also their antiviral and antifungal properties. Moreover, the sea algae contain high levels of minerals, vitamins, proteins, unsaturated fatty acids (Choi et al., 2015). The production of sea algae has increased considerably such that according to the facts and figures provided by Food and Agriculture Organization, the product has increased from 5.13 million tons per year in 1995 to more than 30 million tons a year through aquaculture. About 65% of the produced algae is directly consumed by humans (FAO, 2018). Since the microscopic algae are widely scattered throughout the Persian Gulf and Oman sea, these algae could be an appropriate substitute for the expensive compounds used as aquatic animals' foods (Zheng et al., 2012). Many studies have been conducted on the effect of adding sea algae as a protein resource to the ration on blood parameters and the activity of digestive enzymes of different species of aquatic animals. Zeraatpisheh et al., (2018) the effect of different concentrations of the algae aqueous extract (Sargassum angustifolium) on some blood parameters of rainbow trout (Oncorhynchus mykiss) was investigated. The findings at the end of the experimental period showed that there was a significant difference in the blood parameters between the control group and Sargassum treatments. In addition, in the study by Saligheh Zadeh et al., (2014), the supplementary food effect Spirulina algae on some blood factors and enzyme activities in Mesopotamichthys sharpeyi was investigated. The results of study showed that the fish fed diet containing Spirulina improved blood parameters and digestive enzyme activities compared to control group. The amounts of sea algae to be added to the diet to achieve enhancement of blood parameters and activity of digestive enzyme vary in different species (Choi et al., 2015). Thus, there is a need for further research on the appropriate concentrations of the added algae leading to improvement of blood parameters and activity of digestive enzyme performance.

Laurencia caspica (red algae) is found across the world and there are more than 418 species of this algae. Since this species is found in intertidal zones, it can play a significant role in supplying food, oxygen, and shelter for aquatic animals (Salehipour-Bavarsad et al., 2014). The special properties of this algae are atrichous eukaryotic cell and centriole, sexual reproduction, feeding on foridean starch, having phycobiliprotein in its pigment (accounting for its red color), and conducting photosynthesis without an external endoplasmic network. This alga is a rich source of necessary minerals such as magnesia, calcium, copper, potassium, selenium, zinc, iodine, iron, and vitamins A, B, C, E, and K. Furthermore, this alga contains omega-3 fatty acids and all amino acids required by the human body. However, this alga contains low fat (Gheibi et al., 2019). This alga can prevent the growth of pathogenic bacteria and cancer cells (Derakhshesh et al., 2011; Shafaghi et al., 2016). Thus far, a limited number of studies have been conducted on the application of this alga in aquatic animals' foods. For example, the use of Laurencia (L. caspica) in the diet of rainbow trout led to an increase in the biochemical indices of serum, improvement in the immune system, and also an enhancement of the fish resistance to the acute variant of Aeromonas hydrophila (Kia Daliri et al., 2020). In another study, the use of Laurencia snyderia extract had antibacterial effects on Vibrio harveyi (Dashtiannasab and Yeganeh, 2017).

The goldfish (Carassius auratus) is in the Cyprinidae family and it is similar to common carp (C. carpio) in terms of living and nutrition conditions (Mimeault et al., 2005). The goldfish, as a member of the carp family, is native to East Asia, and they entered Europe for the first time in the 17th century. The fame of goldfish is somewhat drawn from its resistance. The goldfish is an appropriate species to be used for modeling in the studies on nutrition, reproduction, and physiology (Ahmadniaye Motlagh et al., 2021; Jahanbakhshi et al., 2021; Mimeault et al., 2005). Little information is available on the effect of dietary supplementation of Laurencia on physiological responses of aquatic animals. Thus far, no study has been conducted on the effects of red algae (L. caspica) on blood parameters and digestive enzymes parameters of goldfish (C. auratus). Thus, the current study aimed to investigate the effects of dietary red algae (L. caspica) on blood parameters and the digestive enzymes activities of goldfish.

Materials and methods

Fish and fish farming conditions

Goldfish (n= 300) with the initial weight of 6.10±0.1 gr were purchased and transferred from personal farm in Rash city, Iran, to our laboratory. The fish were kept to adapt to the new environment conditions for 14 days, and they were divided randomly into the 400-liter fish tanks after the adaptation period. In the current experiment, four treatments with three replications including the control group (lacking red algae), the second treatment containing five grams per kilograms of red algae, the third treatment containing 10 grams per kilograms of red algae, and the fourth treatment containing 20 grams per kilograms of red algae (Yu et al., 2016). Twenty-five baby goldfish were added to each replication. The fish were fed manually and daily three times a day (at 9 a.m., 1 p.m., and 17 a.m.). The water was changed on a daily basis. To aerate, an air stone was connected to each tank that was connected to the aerating resource. This experiment lasted for 90 days.

Supplying red algae

The red algae (L. caspica) were collected from the southern coasts of Caspian Sea, and transferred to our laboratory. Collecting the algae, they were washed several times using fresh water to eliminate the salt, sand, and other particles. Then, the algae were dried at

50 oC for 24 h. Afterward, they were ground using an electric mill (Tamdoni Jahromi et al., 2021).

Experimental diets and diets preparation

To add different amounts of the algae to the basal diet, at first the amounts of diet for the whole experiment period (90 days) for each treatment was calculated, then all different amounts of algae (5, 10, and 20 gr/kg basal diet) (Tamdoni Jahromi et al., 2021) were sprayed over the food. In addition, gelatin (4 %) was used as binding agent (Pourmozaffar et al., 2019). The dry diets were collected after 48 hours and were kept until being used at 20°C. The commercial carp (Faradaneh, Iran) diet with the approximate analysis of 43% crude protein, 6% crude fat, and 5% crude fiber was used to feed the goldfish.

Blood parameters

At the end of the experiment period, fish were fasted for 24 h. Five fish were selected randomly from each replication. Fish were anesthetized with clove extract at 500 mg/l. Then, blood samples were withdrawn from caudal vein using heparinized syringes, and the hematological parameters including hematocrit, hemoglobin, the total number of white blood cells, and red blood cells were measured. White and red blood cell counts were performed using modified Dacies fluid solution through hemocytometer lam (Firouzbakhsh et al., 2011). Hematocrit was measured through microcentrifuge and using heparinized microhematocrit tubes (Firouzbakhsh et al., 2011). The amounts of hemoglobin in blood were also measured based on hemoglobin cyanomethahemoglobin (Campbell and Ellis, 2013). The blood serum was separated using a centrifuge for 5 minutes (speed: 3000 g, temperature: 4 °C). The amount of total protein based on the Lowry method (Lowry et al., 1951) was measured using bovine serum albumin as the standard sample, and it was measured in the wavelength 750 (nm) using spectrophotometer. Other biochemical parameters were determined using a kit (Pars Azmoon, Iran), and based on the instructions of the manufacturing company. Glucose was measured using oxidase glucose, cholesterol was measured using oxidase cholesterol, and triglyceride was measured using glycerophosphate dehydrogenase methods (Tamadoni Jahromi et al., 2020, 2021).

Digestive enzymes activity

Samples were taken at the end of the experiment period (90 days) to determine the activity of lipase, amylase, and protease. Fish were starved for 48 before taking the samples (Deguara et al., 2003). Then, the intestines (4 fish per replication) were carefully separated, then the contents of the intestines were fully washed using distilled water in order to remove the remaining foods in the intestines (Chong et al., 2002). The samples were kept at -80°C until use. To measure the activity of digestive enzymes, at first, the samples were taken out of freezing conditions and weighed, then they were mixed with 2.0 M sodium chloride with a weight/volume ratio of 1 to 5 using a mixer (Gawlicka et al., 2000). The obtained suspension was centrifuged at 15000 g in 4°C for 20 minutes. Then, the supernatant (replicating this three times for each patient) was collected to assess the enzyme activities. The amylase enzyme activity was measured based on Natalia et al., (2004) method using the evaluation kit of this enzyme according to the instructions of the manufacturer. It was assessed using spectrometer against the blank in the 540 wavelengths. An amylase activity unit was calculated as the amount of released maltose (mg) in 55°C for 3 minutes. Amylase enzyme activity was calculated based on the unit per milligram of protein with three replications for each sample. The protease enzyme activity was evaluated through Moss (1966) method using detection kit concerning the attraction of the 280 nm light.

A protease activity unit was calculated as the amount of tyrosine released in 30 °C for 15 minutes. The activity of protease enzyme was calculated based on the unit per milligram of protein with three replications for each sample. In addition, the extent of lipase enzyme activity was evaluated through Furné et al., (2005) method in 480 nm with commercial kits (Pars Azmoon, Iran). A unit of lipase activity is calculated as the amount of NaOH (0.25 M) consumed to neutralize fatty acids released for 18 hours. Lipase enzyme activity was determined based on the unit per milligram of protein.

Statistical analysis

Results were analyzed statistically using one-way analysis of variance (ANOVA) (Tamadoni Jahromi et al., 2019). A post hoc least significant difference (LSD) test was performed to determine means that were significantly different from each other. Normality was tested using the Kolmogorov–Smirnov test. Statistical difference was accepted when the p value was < 0.05.

Results

Table 1 shows the hematological parameters of goldfish fed by different levels of red algae. There was a significant increase in the total number of red blood cells in treatments fed by red algae compared to the control group (P < 0.05). All experimental groups showed a significant increase compared to the control group in hematocrit (P < 0.05). There was a significant increase in hemoglobin concentration in all groups fed by red algae as compared to the control group (P < 0.05). In addition, there was a significant increase in the number of white blood cells in experimental groups fed by red algae as compared to the control group (P < 0.05). The highest red blood cell numbers, hemoglobin and hematocrit were observed in group fed by 20 grams of red algae per kilogram of diet. Moreover, the highest white blood cell numbers were observed in group fed by 10 grams of red algae per kilogram of diet (P < 0.05).

Table1- Hematological parameters (mean ± S.D) of goldfish (n= 15 in each group) fed with different levels of Laurencia caspica. Different lowercase letters indicate statistically significant differences between different experimental groups (P < 0.05)

Hematology parameters	Control group	5 gr/kg *Laurencia*	10 gr/kg *Laurencia*	20 gr/kg *Laurencia*
White blood cell (10^4 cell/ml)	4 ± 0.20^c	5.23 ± 0.14^b	6 ± 0.17^a	5.24 ± 0.18^b
Red blood cell (10^4 cell/ml)	1.20 ± 0.02^c	1.32 ± 0.03^b	1.34 ± 0.02^b	1.41 ± 0.02^a
Hemoglobin	4.28 ± 0.16^b	5.4 ± 0.14^{ab}	5.65 ± 0.2^{ab}	6 ± 0.24^a

(gr/L)				
Hematocrit (%)	26.30 ± 0.27[bc]	27.88 ± 0.34[b]	28.60 ± 0.41[ab]	29 ± 0.1[a]

Table 2 shows the biochemical parameters of goldfish's fed by different levels of red algae. There was a significant difference between blood biochemical indices such as total glucose and protein in different treatments ($P < 0.05$). There was a significant difference between the triglycerides and cholesterol in groups fed by diets containing red algae and the group fed by control diet ($P < 0.05$). The highest amounts of triglyceride and cholesterol were observed in the control treatment that were 270.86 ± 5.07 and 369.09 ± 6.09 g/dL respectively. The lowest triglycerides concentration between experimental groups was observed in the group fed by 20 grams of red algae per kilogram of diet ($P < 0.05$).

Table 2- Biochemical parameters (mean ± S.D) of goldfish (n= 15 in each group) fed with different levels of Laurencia caspica. Different lowercase letters indicate statistically significant differences between different experimental groups (P < 0.05)

Biochemical parameters	Control group	5 gr/kg *Laurencia*	10 gr/kg *Laurencia*	20 gr/kg *Laurencia*
Glucose (gr/dL)	68.9 ± 6.2	67.31 ± 4.66	68 ± 7.33	67 ± 6.21
Triglyceride (gr/dL)	270.86 ± 5.07[a]	265.26 ± 7[ab]	262.52 ± 8.05[ab]	258.01 ± 5[b]
Cholesterol (gr/dL)	364.09 ± 6.09[a]	345.74 ± 6.16[b]	347.67 ± 5.08[b]	355.16 ± 7.20[ab]
Total protein (gr/dL)	5.6 ± 0.16	6 ± 0.41	5.66 ± 0.31	6.01 ± 0.25

The results of enzyme activities of lipase, amylase, and protease of goldfish fed by different levels of red algae have been presented in Table 3. Adding red algae to goldfish diets led to a significant increase in the activities of digestive enzymes compared to control treatment ($P < 0.05$). The highest activities of protease (290.11 ± 3.20 U/mg protein), amylase (620.26 ± 4.10 U/mg protein), and lipase (19 ± 1.17 U/mg protein) were observed in the treatment containing 20 grams of red algae per kilogram of diet. While the lowest activities of protease enzyme (270.10 ± 5.56 U/mg protein), amylase (585.5 ± 4 U/mg protein), and lipase (15 ± 1.10 U/mg protein) were observed in the control group.

Table 3- Digestive enzyme activities (mean ± S.D) of goldfish (n= 12 in each group) fed with different levels of Laurencia caspica. Different lowercase letters indicate statistically significant differences between different experimental groups (P < 0.05)

Enzyme activity (U/mg protein)	Control group	5 gr/kg *Laurencia*	10 gr/kg *Laurencia*	20 gr/kg *Laurencia*
Lipase	15 ± 1.10[c]	16.80 ± 1[b]	16.46 ± 1.2[b]	19± 1.17[a]
Protease	270.10 ± 5.56[bc]	277.77 ± 8.87[b]	281.33 ± 10.61[ab]	290.11 ± 3.2[a]
Amylase	585.5 ± 4[c]	608.16 ± 6.6[b]	610 ± 3.12[b]	620.26 ± 4.1[a]

Discussion

Thus far, different biological combinations with a wide range of uses like the antibiotic, antiviral, antifungal, and anticancer effects have been identified and extracted from the multicellular algae and many primary or secondary metabolites of these living creatures can be converted into bioactive materials used in pharmaceutical industries (Pereira et al., 2012). One of the important indices involved in the examination of the health status and safety of the fish is the blood parameters that are simpler and less costly compared to other methods (Heydari et al., 2020). The blood parameters in the fish were influenced by different physiological or external factors like food (Rios et al., 2002). According to the results, the significant increase in red blood cells, hemoglobin, and hematocrit in the treatments fed by the algae indicated the positive effect of algae extract on the blood parameters of goldfish compared to the control group. The increase in these parameters is probably due to the effect of antioxidants in the algae extract on the decrease in the hemolysis caused by peroxidation of fats in membranes of red blood cells or the protective effect of polyphenols against the hydrogen peroxide created by the oxidation of red blood cells (Choi et al., 2015). The polyphenols in the herbal compounds can create a complex in combination with the metals and metal ions like iron. Moreover, these materials can get involved in the reactions of iron and other metals. Thus, access to the iron required for hemopoiesis may be facilitated (Kia Daliri et al., 2020). The study by Saligheh Zadeh et al., (2014) showed that hemoglobin increased in the M. sharpeyi fed by Spirulina (5, 10, and 20 grams of Spirulina per kilogram of diet) compared to the control group which aligned with the results of the present study. Such results in Pangasius were also observed in Pangusius fish (Pangusius hypophtalamus) and parrotfish after being fed by the Spirulina (Chelehmal Dezfolinejad et al., 2012; Kim et al., 2013). The access to iron has been enhanced due to the existence of Chlorophyll in Spirulina algae which leads to an increase in hemoglobin (Tangestani et al., 2017). In agreement with these results, the oral administration of hydroalcoholic extract of Laurencia by 5, 10, 20 grams per kilogram of diet increased the number of red blood cells, hematocrit, and hemoglobin compared to the control group (Kia Daliri et al., 2020). The increase in the amounts of the above-mentioned parameters could result from the effects of this alga and the compounds in it on the blood-forming organs and hematopoietic tissue increasing the number of red blood cells and hematocrit (Kim et al., 2013). The white cells are one of the main cells stimulating the innate immune response in fish. Considering the role of white blood cells in the immune system of fish, the change in the number of cells caused by the effect of immune stimuli seems reasonable (Rios et al., 2002). The investigation of the number of white blood cells in the current study indicated the role of the immune stimulus of the algae extract administered in the diet of the goldfish such that the significant increase in the number of white blood cells in the treatments fed by red blood cells compared to the control group show the positive effect of algae on the number of white blood cells of goldfish. Karami et al., (2016) and Zeraatpisheh et al., (2018) reported that the application of an aqueous extract of Sargassum angustifolium in the diet of the common carp (Cyprinus carpio) and rainbow trout (Oncorhynchus mykiss) led to a significant increase in the number of white blood cells compared to the control group which aligned with the results of our experiment. The biochemical parameters of blood were used as a clinical index in monitoring the health status of aquatic animals. Many factors influence the rate of these parameters and make changes in them, and one of these factors is fish nutrition. The results did not show a significant difference in the levels of glucose and total protein in different treatments. However, the level of glucose in the treatments containing algal was less than that of the control group. Heydari et al., (2020) reported that the use of 15 and 25 grams of G. pygmea in the diet of sea bass (L. calcarifer) did not affect the level of glucose and total protein which agreed with the results of our experiment. The results of our experiment showed that the levels of triglycerides and cholesterol in the fish fed by the control group were higher than those of the fish fed by the diets containing red algae powder. The supplementary diet containing algae can influence the metabolism of fish fat (Nakagawa, 1997). Adding Sargassum to the diet of the Asian sea bass (L. calcarifer) diet led to a decrease in blood cholesterol (Heydari et al., 2020). It seems that the phytosterols in the algae prevent the absorption of cholesterol into the digestive system (Sotoudeh, 2020). (Nematipour, Nakagawa, Kasahara, & Ohya, (1988) and Naraghi, Shamsaie Mehrgan, Rajabi Islami, & Hosseini Shekarabi, (2018) reported that the use of diets containing the microalgae Nannochloropsis and Chlorella decreased the cholesterol and triglyceride in fish. This can result from the effect of the algae on the metabolism of liver fats and also the unsaturated omega-3 fatty acids. The decrease in the cholesterol of blood serum caused by the long-chain fatty acids may be related to the decrease in the performance of cholesteryl ester carrying protein and Lecithin–cholesterol acyltransferase (LCAT) (Abbey et al., 1990).

The results showed that the use of different levels of red algae led to an increase in the activities of the digestive enzymes compared to those of the control treatment, and these results were consistent with those of the studies by Akbary and Shahraki, (2020). They reported that feeding Mugil cephalus by Padina algae (Padina astraulis) at 5, 10, and 15gram/kg diet improved the performance of digestive enzymes including amylase, lipase, protease compared to the control treatment. Akbary and Soltanpur, (2019) showed after 60 days of an experiment that the highest activity of amylase, lipase, protease in the Mugil cephalus were observed in the diet containing 15 grams of Sargassum extract (Sargassum ilicifolium). The increase in the activities of digestive enzymes may result from the fact that different herbal additives and seasonings influence the secretion of digestive enzymes and improve the performance of the animals by stimulating the intestinal secretion and producing the enzymes amylase, lipase, protease which lead to the increase in digestibility and accessibility of diet resources (Bita et al., 2019). In another study, the use of Gracilaria algae at levels of 30, 60, and 90 grams per kilogram of food in the diet of Asian sea bass (L. calcarifer) decreased the activities of digestive enzymes such as amylase, lipase, and protease. These researchers reported that the high amounts of non-starch polysaccharides prevent the connections between digestive enzymes and substrate resulting in the decrease in the digestibility of the proteins and fats (Farhoudi et al., 2020).

Overall, the results showed that the red algae significantly improvement of blood parameters (red blood cell, white blood cell, and hematocrit) in comparison to the control group. Additionally, no significant difference in the level of glucose and total protein was found among treatments. However, there was a significant difference in the rate of triglycerides and cholesterol between the treatments fed by red algae extract and the control group. The highest level of protease, amylase, and lipase was observed in the goldfish fed by 20 grams of red algae extract per kilogram of diet. However, there was a significant improvement in blood indices and the activity of digestion enzymes with the use of a diet containing 20 grams of red algae per kilogram of goldfish diet and therefore this diet is recommended.

References

Abbey, M., Clifton, P., Kestin, M., Belling, B. and Nestel, P., 1990. Effect of fish oil on lipoproteins, lecithin: cholesterol acyltransferase, and lipid transfer protein activity in humans. Arteriosclerosis: An Official Journal of the American Heart Association, Inc., 10 (1), 85–94.

Ahmadniaye Motlagh, H., Pourmozaffar, S. and Safari, O., 2021. Effect of garlic extract supplementation on growth performance, nonspecific immunity, and antibacterial activity of skin mucus in goldfish, Carassius auratus. International Journal of Aquatic Biology, 9 (6), 350-359.

Akbary, P. and Shahraki, N., 2020. Effect of dietary supplementation of Padina astraulis (Hauk) extract on biochemical response and digestive enzyme activities of grey mullet, Mugil cephalus (Linnaeus). Iranian Journal of Fisheries Sciences, 19 (4), 2118–2127.

Bita, S., Akbary, P. and Soltanpur, F., 2019. Effect of Sargassum ilicifolium alcoholic extract on growth, feed, body composition and digestive enzymatic activities in Mugil cephalus. Aquatics Physiology and Biotechnology, 6 (4), 61–78.

Campbell, T. W. and Ellis, C. K., 2013. Avian and exotic animal hematology and cytology. John Wiley & Sons.

Chelehmal Dezfolinejad, M., Jahangirzadeh, M., Mesbah, M. and Johari Baboli, M., 2012. The effect of feeding with spirulina (Spirulina platensis) on hematological parameters and immunity system in Pangasius hypophtalamus. Journal of Animal Environment, 4 (2), 25–34.

Choi, Y. H., Lee, B.-J. and Nam, T. J., 2015. Effect of dietary inclusion of Pyropia yezoensis extract on biochemical and immune responses of olive flounder Paralichthys olivaceus. Aquaculture, 435, 347–353.

Chong, A. S. C., Hashim, R., Chow-Yang, L. and Ali, A. B., 2002. Partial characterization and activities of proteases from the digestive tract of discus fish (Symphysodon aequifasciata). Aquaculture, 203 (3–4), 321–333.

Dashtiannasab, A. and Yeganeh, V., 2017. The effect of ethanol extract of a macroalgae Laurencia snyderia on growth parameters and vibriosis resistance in shrimp Litopenaeus vannamei. Iranian Journal of Fisheries Sciences, 16 (1), 210–221.

Deguara, S., Jauncey, K. and Agius, C., 2003. Enzyme activities and pH variations in the digestive tract of gilthead sea bream. Journal of Fish Biology, 62 (5), 1033–1043.

Derakhshesh, B., Yousefzadi, M., Afsharnasab, M., Yeganeh, V. and Dashtiannasab, A., 2011. In vitro antibacterial activities of the marine macroalgae" Laurencia snyderiae" and ‘Sargassum angustifolium’ against human pathogens. Iran South Medical Journal, 14 (1), 17–22.

Farhoudi, A., Sourinejad, I., Nafisi Bahabadi, M., Sajadi, M. M. and Salarzadeh, A., 2020. Dietary supplemental effects of macro algae Gracilaria pygmaea on proximate analysis, apparent digestibility and digestive enzymes activity of Asian sea bass (Lates calcarifer). Aquaculture Sciences, 7 (3), 195–206.

Firouzbakhsh, F., Noori, F., Khalesi, M. K. and Jani-Khalili, K., 2011. Effects of a probiotic, protexin, on the growth performance and hematological parameters in the Oscar (Astronotus ocellatus) fingerlings. Fish Physiology and Biochemistry, 37 (4), 833–842.

Furné, M., Hidalgo, M. C., López, A., García-Gallego, M., Morales, A. E., Domezain, A., Domezainé, J. and Sanz, A., 2005. Digestive enzyme activities in Adriatic sturgeon Acipenser naccarii and rainbow trout Oncorhynchus mykiss. A comparative study. Aquaculture, 250 (1–2), 391–398.

Gawlicka, A., Parent, B., Horn, M. H., Ross, N., Opstad, I. and Torrissen, O. J., 2000. Activity of digestive enzymes in yolk-sac larvae of Atlantic halibut (Hippoglossus hippoglossus): indication of readiness for first feeding. Aquaculture, 184 (3–4), 303–314.

Gheibi, S., Ghaleh, H. E. G., Nezamdoost, N., Shadbad, S. Y., Khamisabadi, M., Moghimi, A., Zamani, A., Bolandian, M. and Motlagh, B. M., 2019. Evaluation the antiproliferative effects of Laurencia caspica extract at the Caspian sea coast on the erythroleukemia K562 cell line. Journal of Marine Medicine, 1 (1), 39–45.

Jahanbakhshi, A., Pourmozaffar, S., Adeshina, I., Mahmoudi, R., Erfanifar, E. and Ajdari, A., 2021. Selenium nanoparticle and selenomethionine as feed additives: Effects on growth performance, hepatic enzymes’ activity, mucosal immune parameters, liver histology, and appetite-related gene transcript in goldfish (Carassius auratus). Fish Physiology and Biochemistry, 47(2), 639-652.

Heydari, M., Zargar, A., Soltani, M., Ebrahimzadeh Mosavi, H. and Morshedi, V., 2020. Effects of different levels of dietary macro algae Gracilaria pygmea on some blood biochemical parameters of Asian seabass (Lates calcarifer). Journal of Animal Environment, 12 (3), 189–196.

Karami, E., Mesbah, M., Mohammadian, T., Hoseini, S. S., Nazari, M. and others, 2016. Effects of aqueous extract of Sargassum angustifolium (Agardh, 1820) on some of the hematological parameters in common carp Cyprinus carpio (Linnaeus, 1758). Journal of Aquatic Ecology, 6 (2), 124–133.

Kia Daliri, M., Firouzbakhsh, F. and Deldar, H., 2020. Effect of oral administration of red alga (Laurencia caspica) hydroalcoholic extract on growth performance, hematological indices and serum biochemistry in rainbow trout (Oncorhynchus mykiss). Journal of Veterinary Research, 75 (3), 328–340.

Kim, S.-S., Rahimnejad, S., Kim, K.-W. and Lee, K.-J., 2013. Partial replacement of fish meal with Spirulina pacifica in diets for parrot fish (Oplegnathus fasciatus). Turkish Journal of Fisheries and Aquatic Sciences, 13 (2). 197-204.

Lowry, O., Rosebrough, N., Farr, A.L. and

Randall, R., 1951. Protein measurement with the Folin phenol reagent. The Journal of biological chemistry, 193 (1), 265–275.

Mimeault, C., Woodhouse, A. J., Miao, X.-S., Metcalfe, C. D., Moon, T. W. and Trudeau, V. L., 2005. The human lipid regulator, gemfibrozil bioconcentrates and reduces testosterone in the goldfish, Carassius auratus. Aquatic Toxicology, 73 (1), 44–54.

Moss, D. W., 1966. Book Review:Practical Clinical Enzymology.

Nakagawa, H., 1997. Effect of dietary algae on improvement of lipid metabolism in fish. Biomedicine & pharmacotherapy, 51 (8), 345–348.

Naraghi, M., Shamsaie Mehrgan, M., Rajabi Islami, H. and Hosseini Shekarabi, S. P., 2018. Effect of dietary supplementation of Nannochloropsis oculata powder on some hematological indices of rainbow trout fingerling. Iranian Journal of Fisheries Sciences, 27 (6), 197-204.

Natalia, Y., Hashim, R., Ali, A. and Chong, A., 2004. Characterization of digestive enzymes in a carnivorous ornamental fish, the Asian bony tongue Scleropages formosus (Osteoglossidae). Aquaculture, 233 (1–4), 305–320.

Nematipour, G. R., Nakagawa, H., Kasahara, S. and Ohya, S., 1988. Effects of dietary lipid level and Chlorella-extract on ayu. Nippon Suisan Gakkaishi, 54 (8), 1395–1400.

Pereira, R., Valente, L. M. P., Sousa-Pinto, I. and Rema, P., 2012. Apparent nutrient digestibility of seaweeds by rainbow trout (Oncorhynchus mykiss) and Nile tilapia (Oreochromis niloticus). Algal Research, 1 (1), 77–82.

Pourmozaffar, S., Tamadoni Jahromi, S., Rameshi, H. and Gozari, M., 2019. Evaluation of some haemolymph biochemical properties and F-cell prevalence in hepatopancreas of white leg shrimp (Litopenaeus vanammei) after fed diets containing apple cider vinegar and propionic acid. Aquaculture Research, 50 (11), 3435-3443.

Rios, F. S., Kalinin, A. L. and Rantin, F. T., 2002. The effects of long-term food deprivation on respiration and haematology of the neotropical fish Hoplias malabaricus. Journal of Fish Biology, 61 (1), 85–95.

Salehipour-Bavarsad, F., Afsharzadeh, S. and others, 2014. Three new records of the genus Laurencia (Ceramiales, Rhodophyta) from the Persian Gulf seashores in Iran. Iranian Journal of Botany, 20 (2), 248–256.

Saligheh Zadeh, R., Yavari, V., Mousavi, S. M. and Zakeri, M., 2014. Effect of dietary supplementation Spirulina platensis on blood, immunological and serum biochemical parameters of Benny fish Mesopotamichthys sharpeyi (Günther, 1874). Iranian Veterinary Journal, 10 (2), 40–46.

Shafaghi, M., Salehzadeh, A. and Moshfegh, A., 2016. Anticancer effects of Laurencia caspica extract on breast cancer T47D cell line. Aquatics Physiology and Biotechnology, 4 (1), 69–83.

Sotoudeh, E., 2020. Growth performance and blood indices of juvenile rainbow trout (Onchorhynchus mykiss) fed diets containing Sargassum cristaefolium and Gracilaria pygmaea extracts. Journal of Applied Ichthyological Research, 8 (1). 80-88.

Tamadoni Jahromi, S., Othman, A.S. and

Rosazlina, R., 2019. Morphometrics and mitochondrial DNA genes analysis suggest a new species of Penaeus (Crustacea: Penaeidae) from the Persian Gulf. Biochemical genetics, 57 (2), 193-213.

Tamadoni Jahromi, S., Pourmozaffar, S., Rameshi, H., Gozari, M. and Nahavandi, R., 2020. Evaluation of hemolymph biochemical properties, clearance rate, bacterial microbiota and expression of HSP genes of gulf pearl oyster Pinctada radiata in response to salinity changes. Fisheries Science, 86 (6), 1055-1065.

Tamadoni Jahromi, S., Pourmozaffar, S., Jahanbakhshi, A., Rameshi, H., Gozari, M., Khodadadi, M., Sohrabipour, J., Behzadi, S., Bazrkar, N., Nahavandi, R. and others, 2021. Effect of different levels of dietary Sargassum cristaefolium on growth performance, hematological parameters, histological structure of hepatopancreas and intestinal microbiota of Litopenaeus vannamei. Aquaculture, 533, 736130.

Tangestani, N., Morshedi, V., Nafisi Bahabadi, M., Azodi, M., Farhodi, A. and Sotoudeh, E., 2017. effects of partial substitution of macro algae Gracilaria pygmaea with fish meal on some blood of Asian sea bass (Lates calcarifer). Journal of Animal Physiology and Development, 10 (2), 37–51.

Yu, Y. Y., Chen, W. D., Liu, Y. J., Niu, J., Chen, M. and Tian, L. X., 2016. Effect of different dietary levels of Gracilaria lemaneiformis dry power on growth performance, hematological parameters and intestinal structure of juvenile Pacific white shrimp (Litopenaeus vannamei). Aquaculture, 450, 356–362.

Zeraatpisheh, F., Firouzbakhsh, F. and Khalili, K. J., 2018. Effects of the macroalga Sargassum angustifolium hot water extract on hematological parameters and immune responses in rainbow trout (Oncohrynchus mykiss) infected with Yersinia rukeri. Journal of Applied Phycology, 30 (3), 2029–2037.

Zheng, K., Liang, M., Yao, H., Wang, J. and Chang, Q., 2012. Effect of dietary fish protein hydrolysate on growth, feed utilization and IGF-I levels of Japanese flounder (Paralichthys olivaceus). Aquaculture Nutrition, 18 (3), 297–303.