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# **Research Article**

# Dietary administration of Sargassum angustifolium and Gracilaria pulvinata extracts affect antioxidant enzyme activities and Lactobacillus bacterial population in intestine of rainbow trout (Oncorhynchus mykiss) fry

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Received: January 2019 Accepted: January 2020

#### **Abstract**

This study investigated the effect of Sargassum angustifolium and Gracilaria pulvinata extracts on the growth performance, body composition, antioxidant status and gut microbiota of rainbow trout (Oncorhynchus mykiss) fry. For this purpose, 540 fry of rainbow trout (initial weight 0.23±0.04g) were distributed in 12 fiberglass tanks. Fish were fed with experimental diets, containing 500mg kg<sup>-1</sup> of Sargassum extract (SA), 500mg kg<sup>-1</sup> of Gracilaria extract (GL) and 250mg kg<sup>-</sup> Sargassum+250mg kg<sup>-1</sup> Gracilaria extract (SA+GL). The control diet was a commercial diet without seaweed extract. At the end of the experiment growth and feeding performance, including final weight, specific growth rate (SGR), weight gain (WG) and feed conversion (FCR) in all treatments were not significantly different (p>0.05). Whole body protein, ash and moisture contents of fish were not significantly affected by dietary macroalgae extracts (p>0.05). However, lipid content was significantly lower in fish fed with extract og Gracilaria and Sargassum compared to control (p < 0.05). The seaweed extract-fed fish utilized efficiently the body lipid and suppressed weight loss of the body during starvation. No significant difference (p>0.05) in serum total protein, creatinine alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and blood superoxide dismutase (SOD) activity was observed in all treatments. Whereas serum albumin, glutathione peroxidase (GPX) and catalase specific activity were higher in SA, GL and SA+GL treatments compared to the control group (p<0.05). ACH50 level was significantly (p<0.05) increased in GL and SA+GL treatments as compared to SA and control group. The addition of Sargassum and Gracilaria extracts to feed did not affect total bacterial population but increased Lactobacillus bacteria levels (p<0.05) in the intestine. These results revealed potential antioxidant enhancing and health-promoting effects of dietary Sargassum angustifolium and Gracilaria pulvinata extracts.

**Keywords:** Macroalgae extract, Immunostimulant, Bacterial population, Antioxidant status, Rainbow trout

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#### Introduction

Rainbow trout, Oncorhynchus mykiss, is a fast-growing species and one of the most important fish species that is commercially farmed in many countries (FAO, 2014). Culturing this species in both semi-intensive or intensive systems lead to a stressful condition that negatively affects fish health and immune system and increases susceptibility to infections (Trenzado et al., 2007; Hoseinifar et al., 2015). A practical approach for resolving the issue is by potentially stimulating the immune system of the host animal by inclusion of natural immunostimulants (Wang et al., 2017). Compounds with immunostimulant effect can improve health status and disease resistance.

Seaweeds are good source of protein, lipid, pigments, minerals and vitamins (Matanjun et al., 2009) and many studies focused on administration of dietary seaweeds reporing positive results on growth, feed utilization, lipid metabolism, controlling disease and disease resistance of various fish and shrimp species (Castro et al., 2004; Araújo et al., 2016; Peixoto et al., 2016: Thanigaivel etal.. Sotoudeh and Jafari 2017; Morshedi et al., 2018). Furthermore, seaweeds and their various derived substances are of valuable sources bioactive can compounds, which increase immune responses and be used as dietary immunostimulant in aquaculture (Jiménez-Escrig et al., 2011; Araújo et al., 2016; Dashtiannasb and Yeganeh, 2017). Seaweeds are identified and grouped into three different class, including brown algae (Phaeophyta), red algae (Rhodophyta) and green algae (Chlorophyta). *Gracilaria* spp. Sargassum are red (class: spp. Rhodophyta) and brown algae. respectively which contain relatively high level of essential amino acids, essential fatty acids and minerals (Matanjun et al., 2009). Several studies focused on effects of Sargassum extract on immune resistance in black tiger shrimp, *Penaeus monodon* (Immanuel et al., 2012), flathead grey mullet, Mugil cephalus (Kanimozhi et al., 2013), tilapia, Oreochromis niloticus (Isnansetyo et al., 2016) and Asian sea bass. Lates calcarifer Bloch (Yangthong et al., 2016). In general, these findings suggested that Sargassum extract can be used as an immunostimulant in fish and shrimp. There is also evidence that Gracilaria verrucosa enhanced some innate immune parameters in black tiger shrimp (Maftuch and Risjani, 2012) and dietary *Gracilaria* spp. supplementation in European seabass (Dicentrarchus labrax) led to an antioxidant capacity enhancement (Peixoto et al., 2016).

Gut microbiota has many functions for health of the host and is considered as an integral component of the host. Recently some researchers proposed that microbiota change in the intestine of fish may affect host immune functions (Han *et al.*, 2018). However, no study focused on administration effects of dietary seaweeds on gut microbiota in fish.

The objective of this research was to evaluate the effects of hydroalcoholic extract of *Sargassum angustifolium* and *Gracilaria pulvinata* as dietary supplements on growth performance, blood index, chemical composition, antioxidant status and gut microbiota of rainbow trout (*Oncorhynchus mykiss*) fry.

#### Materials and methods

# Fish and experimental diets

Five hundred and forty rainbow trout fry were obtained from Shahid Motahary Coldwater Fish Genetic and Breeding Research Center, Yasouj, Iran transferred to fish culture and of Persian Gulf experimental lab University, Bushehr Province, Iran. The fishes were acclimatized experimental condition for two weeks. After this period, fish (0.23±0.04g) were randomly stocked into 12 square fiberglass tanks (20 litter) in triplicate and fed for 6 weeks. Following the feeding trial, the fish were kept in the tanks and starved for (Nakagawa, 2004). Body weight loss and reduction of body lipid and protein monitored. were Each replicate consisted of 45 fish in a tank with flowthrough water system (31/min). During the trial (60 days) photoperiod was 12:12h (light: dark) and temperature, dissolved oxygen and pH were  $14.7\pm0.5^{\circ}$ C,  $7.5\pm0.5$ mg L<sup>-1</sup> and  $7.2\pm0.2$ , respectively.

## Diet preparation

Before preparation of the experimental diet, the brown seaweed Sargassum

angustifolium and the red seaweed Gracilaria pulvinata were collected from Persian Gulf coast of Bushehr, Iran. After taxonomic identification. they were washed and dried at 50°C for 24h, then powdered and extracted as described by Thanigaivel et al. (2015). Then a commercial feed (Beyza, Iran) was considered as basal diet (control) and other experimental diets were prepared by spraying macroalgae solutions extract uniformly on the feed, 500mg kg<sup>-1</sup> of Sargassum extract (SA), 500 mg kg<sup>-1</sup> of *Gracilaria* extract (GL) and 250mg kg<sup>-1</sup>of Sargassum + 250mg kg-1 Gracilaria extract (SA+GL, Table 1). The control group was fed by basal macroalgae diet without extract. Prepared diets were kept in plastic bags at -4°C until used. The feeding rate was 10% of body weight in equal rations at each feeding time, 08:00, 12:00, 16:00 and 20:00 (Sotoudeh et al., 2016).

Table 1: Proximate composition of the basal commercial diet.

commercial dict.				
Proximate composition (dry				
matter basis)				
Dry matter (%)	90.00			
Crude protein (%)	50.00			
Crude lipid (%)	13.50			
Fiber (%)	1.70			
Energy (kj/g)	17.99			

## Growth Performance

To measure growth performance, weight and length of fish in each tank was monitored in two weeks and at the end of the experiment following a 24 hours starvation. Growth performance and survival rate of fingerlings were calculated using the following formula (Hamza *et al.*, 2008), FCR (feed

conversion ratio)= $F/(B_t-B_0)$ , **SGR** (specific growth rate)=(Ln W<sub>t</sub> -Ln  $W_0$ )×00/t, WG (weight gain)= $W_t$ - $W_0$ , **CF** K (condition or factor)=100×(Wt/TL<sup>3</sup>), viscerosomatic  $(VSI)=100\times(Wv/Wt)$ , index survival rate= $100 \times (N_t / N_0)$ . Where: F: relative food intake (g), B<sub>t</sub> and B<sub>0</sub>: final and initial fish biomass (g),  $W_t$  and  $W_0$ : final and initial body weight (g), Wv: viscera weight, t: time of rearing (days), TL: total length, N<sub>t</sub> and N<sub>0</sub>: final and initial fish number.

# Sample collection

Five specimens were randomly selected from each replicate and anesthetized with clove solution at 30 mg/L (Velisek *et al.*, 2005) for blood sampling, proximate analysis and bacteriology. The blood samples were obtained from the caudal vein and divided into two parts.

## Blood biochemistry parameters

For blood biochemistry parameters, the samples were transferred to heparinized tubes and the remainings to nonheparinized tubes for serum isolation. The samples were centrifuged (3000×g for 15 min at 4°C) and the obtained serums were stored at -80°C until use. Biochemical parameters, including total protein (g dl<sup>-1</sup>), albumin (g dl<sup>-1</sup>), triglyceride (mg dl<sup>-1</sup>) and glucose (mg dl<sup>-1</sup>) in serum were analyzed using a commercial kit (Pars Azmun Ltd., Iran) and a spectrophotometer (Hitachi Ltd., Tokyo, Japan) was used for (Raeeszadeh measurement and Beheshtipour, 2018).

Alanine aminotransferase (ALT, IU I<sup>-1</sup>) and Aspartate aminotransferase (AST, IU I<sup>-1</sup>) were measured according to Huang *et al.* (2006) method. Alkaline phosphatase (ALP, IU I<sup>-1</sup>) was measured according to Bessey *et al.* (1946).

Alternative complement pathway (ACH50) activity was estimated as described by Tort et al. (1996) and ACH50 units were defined as the concentration of plasma giving 50% haemolysis of rabbit red blood cell, as target cells, in the presence of ethylene glycol tetraacetic acid (EGTA, Sigma) and Mg<sup>2+</sup>. Briefly, fifty microlitres of plasma were used for each analysis, and samples were incubated at 37°C for 60 min. They were then centrifuged and the extent of haemolysis was estimated by measuring the optical density of the supernatant at 414nm using an ELISA reader (Epoch, BioTek, Highland Park, VT, USA). The results are presented as ACH UmL<sup>-1</sup>.

## Antioxidant enzymes activity

For evaluation of antioxidant enzymes the liver samples activity, obtained from nine fish per treatment. First, the samples were homogenized in 9 volumes of 0.05M phosphate buffer pH=6.6 containing 1% Triton X-100, a tissue homogenizer centrifuged at 10000g at 4°C for 30 min. The obtained supernatants were kept at -80°C until further analysis. All enzyme preparations were carried out on ice. Dilution of the sample was done when required. Superoxide dismutase (SOD) activity was measured following the method described by McCord and Fridovich (1969). Catalase (CAT) activity was determined following the method described by Beers and Sizer (1951). The glutathione peroxidase (GPX) activity was measured using a GPX Assay Kit (Nanjing Jiancheng Bioengineering Institute, China) according to manufacturer's instructions.

## Chemical analysis

An initial sample of five fish was taken for proximate analysis. At the end of the experiment whole body of five fish per tank were homogenized in a blender. All analyses were performed in triplicate. Analyses of crude protein, moisture and ash of whole body of fish were performed according to standard procedures (AOAC, 2000). Total lipids in the carcass of fish was analyzed according to Folch et al. (1957) after extraction with chloroform-methanol (2:1). Crude protein value was analyzed according to the Kjeldahl method using an Auto Kjeldahl System (Kjeltec Auto Analyzer; FOSS, Hillerød, Denmark). moisture of samples gravimetrically determined by drying at 110°C and ash content of fish was determined after incineration at 550°C for 6h.

## Intestinal microbiota analysis

Total bacterial population and *Lactobacillus* bacteria levels were determined at the end of the experiment (n=3 fish per tank). The fish were euthanized by overdose (200mg 1<sup>-1</sup> water for 10 min) of tricaine methane sulphonate (MS222) and the skin was

washed in a solution of 0.1% benzalkonium chloride. Intestinal tissue samples were then excised and washed with PBS and homogenized in sterile saline (0.85% (w/v) NaCl).

The enumeration of *Lactobacillus* population was performed following the procedure described by Ferguson *et al.* (2010).

## Statistical analysis

Prior to statistical analyses, all data were tested for normality of distribution using Kolmogorov-Smirnov test. Then the data were subjected to one-way variance (ANOVA) analysis of followed by Duncan's multiple range test. In case of all statistics analysis mean values were considered significantly different at p<0.05. Data were shown as means ± standard deviation. All data were analyzed using SPSS 17 for Windows software (SPSS, Chicago, IL, USA) and the figures were prepared by Microsoft Excel.

#### **Results**

## Fish growth performance

The effects of dietary supplementation Sargassum angustifolium Gracilaria pulvinata hydroalcoholic extracts on mean body weight of fry during different experimental periods are present in Fig. 1. The average fish weight increased along the trial. Fry body weight at 28 and 42 days was not different among treatment groups (p>0.05). The results regarding growth performance and feed utilization of rainbow trout fry are present in Table 2. After 6 weeks trial, no significant difference (p>0.05) observed between

treatments and control group in case of growth and feed utilization parameters (FCR, SGR, WG, CF).

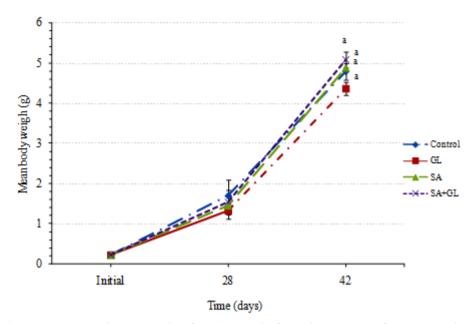


Figure 1: Mean body weight evolution for 6 weeks in fry rainbow trout fed on experimental diets (n=3). Values not sharing a common superscript were significantly different (p<0.05). Error bars show standard deviation. GL: Gracilaria, SA: Sargassum, GL+SA: Gracilaria+Sargassum.

Table 2: Growth performance, feed utilization and survival of rainbow trout fed diets supplemented with Sargassum angustifolium and Gracilaria pulvinata extracts for 6 weeks. Data are presented as mean ± standard deviation (n =3). Data assigned with different superscripts in a column differed significantly at p<0.05. FW: Final weight, FCR: feed conversion ratio, SGR: specific growth rate, WG: weight gain, CF: condition factor, VSI: viscera index, GL: Gracilaria, SA: Sargassum, GL+SA: Gracilaria + Sargassum.

Gracia ia Sangassanii							
Treatments	FW (g)	FCR	SGR (% day <sup>-1</sup> )	WG (%)	CF	VSI (%)	Survival (%)
Control	4.79±0.4	1.71±0.48	6.36±1.47	174.5±53.0	1.24±0.26	7.98±3.9	100
SA	$4.36\pm0.6$	$1.06\pm0.14$	8.11±1.17	241.2±62.9	$1.35 \pm 0.05$	$9.20\pm0.45$	100
GL	$4.88\pm0.8$	$1.22\pm0.00$	$7.33\pm0.11$	$200.2 \pm 5.1$	$1.32\pm0.14$	9.44±3.16	100
GL +SA	$5.08\pm1.3$	$1.29\pm0.07$	$7.05\pm0.24$	$188.0 \pm 10.5$	$1.29\pm0.09$	$8.83\pm2.60$	100

#### Proximate body composition

The body composition of rainbow trout fry fed with extract of *Sargassum* and *Gracilaria* macroalgae in different experimental diets are shown in Table 3. Regarding, protein, ash and moisture no significant difference was observed between treatments and control group after six weeks feeding and 10 days

starvation (p>0.05). Whereas lipid was significantly lower in fish fed with extract *Gracilaria* and *Sargassum* compared to control at the end of six weeks feeding (p<0.05).

Fig. 2 shows percentage loss of body weight, whole-body protein and lipid of rainbow trout fry after 10 days of starvation. Body weight loss was

significantly higher in control group (P < 0.05) in comparison to other groups. The starvation significantly depressed the lipid content in fish fed with seaweed extract (p<0.05). While

consumption of the body protein during starvation was slightly suppressed (not significantly) by the seaweed extract supplementation.

Table 3: Proximate chemical analysis (% on dry matter basis) of whole body of rainbow trout fed diets supplemented with Sargassum angustifolium and Gracilaria pulvinata extracts for 6 weeks. Data are presented as mean±standard deviation (n=3). Data assigned with different superscripts in a column differed significantly at p<0.05. C: control group, GL: Gracilaria, SA: Sargassum, GL+SA: Gracilaria+Sargassum.

Treatments	Chemical characteristics					
	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)		
Initial	12.3	6.1	2.7	74.8		
After 6 weeks feeding						
Control	$14.6 \pm 0.71$	$8.1\pm0.13^{a}$	$2.1\pm0.07$	74.5±2.04		
GL	$15.2 \pm 1.4$	$6.6\pm0.73^{b}$	$2.3\pm0.32$	$72.4 \pm 1.5$		
SA	$15.3\pm0.36$	$6.7\pm0.61^{b}$	$2.1\pm0.28$	$71.8 \pm 1.44$		
GL+SA	15.6±0.77	$6.5\pm0.55^{b}$	2.1±0.06	72.5±1.85		

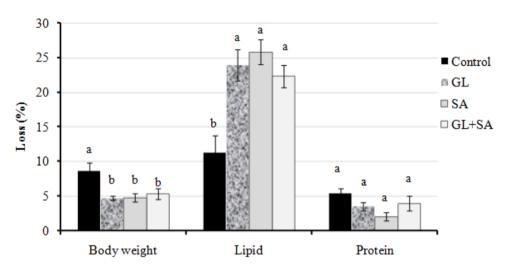


Figure 2: Mean effect of Sargassum and Gracilaria extracts supplementation to the diet on loss in body weight, body lipids (lipids) and body protein (protein) of the rainbow trout fry after 10 days starvation (n=3). Values not sharing a common superscript were significantly different (p<0.05). Error bars show standard deviation. GL: Gracilaria, SA: Sargassum, GL+SA: Gracilaria+Sargassum.

## Biochemical parameters of blood

The blood biochemistry parameters of rainbow fry fed with different experimental diets are shown in Table 4. No significant difference in serum total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) was observed among treatments (*p*>0.05). Fish fed on diets supplemented with *Sargassum* and *Gracilaria* extracts especially mix *Sargassum* and

Gracilaria treatment (SA+GL) showed higher albumin contents significantly (*p*<0.05). The serum glucose and triglyceride levels decreased significantly in fish fed with diets containing *Sargassum* and *Gracilaria* 

(p<0.05). ACH50 level increased treatments as compared with SA and control groups (p<0.05).

Table 4: Blood biochemistry parameters of rainbow trout fed diets supplemented with Sargassum angustifolium and Gracilaria pulvinata extracts for 6 weeks. Values are presented as mean±standard deviation (n =3). Data assigned with different superscripts in a column differed significantly at p<0.05. ALB: albumin, GLU: glucose, ACH50: alternative complement pathway, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, GL: Gracilaria, SA: Sargassum, GL+SA: Gracilaria+Sargassum.

Treatment	Total Protein (g dl <sup>-1</sup> )	ALB (mg dl <sup>-1</sup> )	TG (g dl <sup>-1</sup> )	GLU (mg dl <sup>-1</sup> )	ALT (IU l <sup>-1</sup> )	AST (IU l <sup>-1</sup> )	ALK (IU l <sup>-1</sup> )	ACH50 (U ml <sup>-1</sup> )
Control	3.81±0.1	1.16±0.04°	232.6±20.0 <sup>a</sup>	57.7±2.5 <sup>a</sup>	85.0±7.9	1111.6±17.5	459.0±69.9	71.1±4.1 <sup>b</sup>
SA	$3.58\pm0.2$	$1.50\pm0.1^{b}$	$208.5\pm7.1^{b}$	$39.7 \pm 3.5^{b}$	89.6±8.9	1124.3±32.0	465.6±66.1	$71.1\pm4.2^{b}$
GL	$3.75\pm0.1$	$1.91\pm0.2^{a}$	$164.0\pm8.5^{c}$	$44.3 \pm 5.7^{b}$	95.6±8.5	1115.0±21.9	531.3±103.5	81.9±6.1 <sup>a</sup>
GL+SA	$3.92\pm0.2$	$2.03\pm0.1^{a}$	$202.0\pm27.6^{b}$	$39.0\pm3.0^{b}$	98.9±5.8	1161.5±31.4	533.0±11.1	$81.3\pm3.2^{a}$

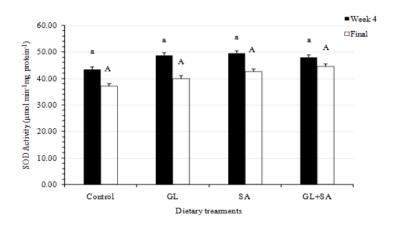
# Antioxidant enzyme activities

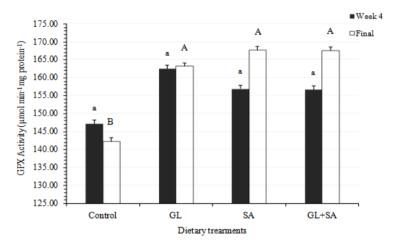
Effects of hydroalcoholic extract of Sargassum and Gracilaria macroalgae on antioxidant enzymes activity are shown in Fig. 3. The statistical analysis of results revealed no difference in superoxide dismutase (SOD) activity among the treatments and control group at weeks 4 and at the end of the experiment (p>0.05). However, regardless of inclusion level, feeding on Sargassum and Gracilaria macroalgae supplemented diet significantly increased glutathione peroxidase (GPX) activity compared with the control group at the end of experiment (p<0.05). Similarly these revealed feeding on Sargassum and Gracilaria macroalgae supplemented diets increased (p<0.05) catalase (CAT) specific activity significantly among the

treatments and control group at week 4 and the end of the experiment (p<0.05).

## Intestinal microbiota

The effect of hydroalcoholic extract of Sargassum and Gracilaria macroalgae on total bacterial population Lactobacillus bacteria levels of rainbow trout fry are shown in Fig. 4. The results revealed that feeding Sargassum and Gracilaria macroalgae supplemented diets increased Lactobacillus bacteria levels significantly compared with the control group at the end of experiment while total bacterial (p<0.05),population was not affected by dietary extract of Sargassum and Gracilaria macroalgae (p>0.05).





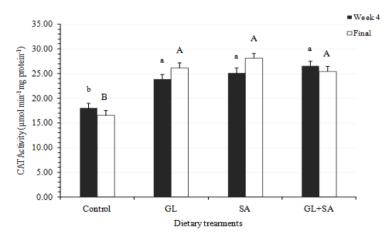


Figure 3: Mean activity of antioxidant enzymes of rainbow trout fry fed the experimental diets at fourth week and at the end of the experiment (n=3). Values not sharing a common superscript were significantly different (p<0.05). Error bars show standad deviation. GL: *Gracilaria*, SA: *Sargassum*, GL+SA: *Gracilaria*+ *Sargassum*.

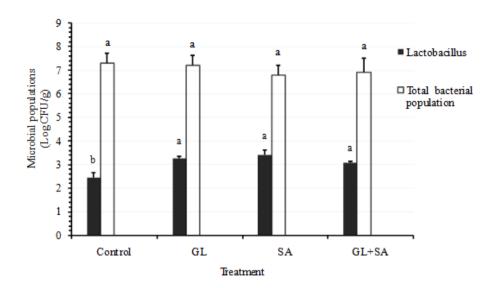


Figure 4: Mean total bacterial population and lactobacillus bacteria levels in intestine of rainbow trout fed the experimental diets at the end of the experiment (n=3). Values not sharing a common superscript are significantly different (p<0.05). Error bars show standard deviation. GL: *Gracilaria*, SA: *Sargassum*, GL+SA: *Gracilaria*+Sargassum.

#### **Discussion**

To our knowledge, this study is first inclusion attempt to evaluate hydroalcoholic extract of Sargassum angustifolium and Gracilaria pulvinata macroalgae in rainbow trout fry diet. The present results revealed no significant difference in growth performance between the treatments and control group at the end of 42 days of the experiment (p>0.05). Similar results are reported in olive flounder (Paralichthys olivaceus) fed with Sargassum fusiforme (Kim et al., 2014) and European seabass (Dicentrarchus labrax) fed with Gracilaria spp. (Peixoto et al., 2016).

Regarding protein, ash and moisture no significant difference was observed between treatments and control group after 6 weeks feeding and 10 days starvation (p>0.05). Similar results are

described by Silva et al. (2015) and Peixoto al.(2016)for body Nile composition of tilapia (Oreochromis niloticus) and seabass fed with Gracilaria vermiculophylla and Gracilaria spp. respectively. Body lipid content of rainbow trout increased in fish fed with Gracilaria and Sargassum extracts compared to control. It is reported that the body lipid decreases in rainbow trout when fed with Sargassum ilicifolium meal (10% of basal diet). It reflects this fact that this macro algae fat are likely to play an important role in the energy of rainbow trout and are more likely to accumulate in meat. Also rainbow trout species uses unsaturated energy fatty acids for needs (Zamannejad et al., 2016). In the peresent study seaweed extract-fed consumed efficiently groups reserved lipids and suppressed body

protein consumption during starvation. As a result, body weight loss could be minimized. The phenomenon starvation for 10 days after the feeding containing Gracilaria Sargassum extract resulted in low body weight loss was caused by preferential lipid mobilization to energy suppression of body protein consumption. It is also showed that lipid metabolism in fish is affected by supplementation dietary of algae (Nakagawa, 1997). Feed containing Spirulina sp. depress triglyceride accumulation in muscle and intraperitoneal fat body of 2-year old red sea bream (Pagrus major) (Mustafa et al., 1994). They found that dietary Spirulina elevate hepatic carnitine palmitoyltransferase activity and hepatic carnitine level which play important roles in P-oxidation of fatty acids. Nematipour et al. (1987) reported that dietary 2% Chorella-extract reduce lipid levels in muscle, liver and adipose tissue of ayu (Plecoglossus altivelis) by stimulating lipolytic hormones and/or influencing the adipose tissue structure. However, the effect of algae on composition of the carcasses seems to be related to their nutritional value and the level of dietary inclusion. Other influential factors include fish species, age, size and experimental protocol (Güroy et al., 2007; Dantagnan et al., 2009; Ergün et al., 2009).

Total protein of serum is one of the components of nonspecific immune system of fish that is affected by immunostimulants (Siwicki *et al.*, 1994). In this study, fish fed with

dietary Sargassum and Grasilaria showed no effect on total serum protein. Similarty, total protein in Nile tilapia were not affected by Ulva lactuca and Pterocladia capillaceaas (Khalafalla and El-Hais. 2015). Albumin is one of the main serum proteins that is associated with immune status, such as globulin, and can be used as indicator of health and immune status (Kumar et al., 2005). In the present study dietary Sargassum and Gracilaria elevated albumin contents in rainbow trout.

Results of the present study showed on Gracilaria that feeding and Sargassum supplemented diet did not affect ALT, AST and ALP. The serum glucose and triglyceride level were decreased in fish fed diets containing Sargassum and Gracilaria significantly (p<0.05). Hypoglycemic plants increase insulin secretion and prevent absorption of glucose from the intestine and produce glucose from liver. With increased stimulation and glucose metabolism by the muscle, insulin reduces blood glucose concentrations. Several studies reported that seaweeds, such as Ecklonia stolonifera (Iwai, 2008), Ascophyllum nodosum Fucus vesiculosus (Lordan et al., 2013), and Gracillaria arcuata (Akbary et al., 2020) have anti-diabetic effects and greatly reduce blood glucose levels. However, it is reported that triglyceride in Nile tilapia and dusky kob, Argyrosomus japonicus, were not affected by dietary Ulva lactuca and Ulva inclusion, respectively sp.

(Khalafalla and El-Hais 2015; Madibana *et al.*, 2017).

The complement activity (ACH50) is an important component of non-specific immune system protecting fish from pathogens (Peixoto et al., 2016). In our study ACH50 level increased in GL and SA+GL treatments as compared with SA and control groups. Similarly supplementation of Gracilaria vermiculophylla and Ulva spp. meal in diet for rainbow trout and Nile tilapia enhanced complement activity respectively (Araújo et al., 2016: Valente et al., 2016). Araújo et al. (2016) stated that algae compounds, polysaccharides, such mainly carrageenan, alginates, fucoidan and βstimulate immunological glucan, response and play important role in disease resistance in several species. including rainbow trout.

It is well-stablished that free radicals are produced during normal processes body (Yang et al.. Antioxidant enzymes (i.e SOD, CAT and GPX) are responsible for protecting fish against oxidative stress (Zhang et al., 2013). Therefore, they have vital roles in maintaining health (Santacroce et al., 2012). In the present study, there was no significant difference in SOD activity compared all treatments with the control. These findings were in accordance with those of previous studies which suggest, SOD activity in liver tissue is not significantly altered in rabbitfish (Siganus canaliculatus) after feeding Gracilaria lemaneiformis (Xu et al., 2011). However, activity of CAT and GPX in liver tissue homogenates

showed significant increase in fish fed with Sargassum and Grasilaria extracts. In one study, supplementation of European seabass diet with different levels of Gracilaria spp. increased the activity of antioxidant enzymes such as GPX in liver but had no effected on CAT (Peixoto et al., 2016). Red seaweeds, such as *Gracilaria* spp. are good source of antioxidants selenium (Devi et al., 2011) which functions as GPX cofactor (Rotruck et al., 1973). In this case it is possible that Gracilaria spp. due to Selenium increment, the effect on over GPX activity (Peixoto et al., 2016).

The intestinal microbiota of fish plays a key role in physiology and function of the gastrointestinal tract as well as stimulation of immune response of the host (Ringø et al., 2014). It is well documented that manipulating intestinal microbiota increases diseases resistance and improves the health of fish (Ringø et al., 2010a). To our knowledge there are no study to reveal the effects of macroalgae on intestinal microbiota in fish. The results also revealed a notable increase Lactobacillus bacteria levels in fish fed Gracilaria with Sargassum and extracts, while total bacterial population was not affected by dietary extract of Sargassum and Gracilaria macroalgae. Studies are done to increase the level of bacteria intestinal lactic acid in microbiota following administration of dietary immunostimulant such probiotics (Azimirad et al., 2016). Lactic acid bacteria are generally known to be the beneficial component

of fish intestinal microbiota, and producing lactic acid, bacteriocins and other antagonistic compounds affect health of the host (Ringø *et al.*, 2010b; Ringø *et al.*, 2014).

In conclusion, the present results revealed beneficial effects of dietary Sargassum angustifolium and Gracilaria pulvinata extracts on activity of antioxidant enzymes and intestinal microbiota in rainbow trout. However. additional studies are requared to evaluate effects of this algae on resistance of fish against bacterial infection and following stress conditions.

## Acknowledgments

The authors would like to thank Raana Bahadori, Zeinab Eshaghnejad, Heidari Zahra Fatemeh and Yousofzadeh Dolabi for their cooperation and assistance throughout the research. Funding for the present study was provided by Persian Gulf University.

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