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Growth performance, plasma parameters and liver antioxidant enzymes activities of Rainbow trout (*Oncorhynchus mykiss*) juvenile fed on *Spirulina platensis* extract

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Abstract

The purpose of this study was to evaluate the physiological responses of Rainbow trout fed on diets supplemented with methanolic extract of *Spirulina platensis.* 150 fish with a mean initial weight of 12.6±0.3 g were randomly divided into five groups. The juveniles were fed for 8 weeks with the diets containing 0, 250, 500, 1000 and 2500 mg of *S. platensis* extract per kg of diet. After eight weeks, 12 fish from each treatment were subjected to confinement stress. It has been shown that 250 mg of the algae extract per kg of diet resulted in the highest final weight and lowest feed conversion ratio in fish. There was no significant difference between treatments in fat, protein and moisture contents. The results showed that three hours after the air exposure and confinement stress, minimum (2.42 mg dl^{-1}) and maximum (6.18±2.5 mg dl^{-1}) levels of plasma cortisol were observed in 2500 and 500 mg kg^{-1} treatments, respectively. Moreover, the minimum (66±14 mg dl^{-1}) and maximum (168±18 mg dl^{-1}) level of glucose has been observed in 0 and 500 mg kg^{-1} treatments, respectively. After eight hours, Plasma cortisol and glucose have showed the highest level in 500 mg kg^{-1} treatment. There was no difference in lysozyme and catalase enzyme activities, but higher Spirulina extract in diet led to an increase in superoxide dismutase enzyme activity in the liver of Rainbow trout. This study suggests that addition of 250 mg kg^{-1} of Spirulina extract to Rainbow trout diet can improve growth performance and stress condition.

Keywords: Microalgae extract, Antioxidant defense, Superoxide dismutase, Confinement stress.

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Introduction

Aquaculture, as the fastest growing food production industry in the world, annually produces 106 million ton of food (FAO, 2017). Culturing excessive numbers of fish in enclosed spaces has negatively affected the health of fish resulting in increased susceptibility to diseases and stress (Sakai, 1999). Despite numerous studies done on the stress responses of fishes, the relationship between diet composition, fish stress and immune response has received little attention (Li *et al.,* 2012). Fish farms need to add additives in the feed to alleviate the adverse effects on animals. Due to the restrictions placed on the use of chemicals such as antibiotics and hormones in feeding aquatic animals, which will be later consumed by humans, the use of natural substances such as those derived from plant and algae in fish food processing has been increased. Growth and immune stimulants of plant origin have several advantages over chemically produced ones including availability, less harm to the environment and animals, and the possibility of production at a low cost (Francis *et al.,* 2001).

Microalgae such as Spirulina are considered rich sources of vitamins, minerals, polysaccharides and fatty acids (Lee *et al.,* 2013). Microalgae have many other valuable properties including species diversity, abundance in nature, adaptability to different environments, easy cultivation (Nahas *et al.,* 2007; Wang *et al.,* 2010) and bioactive compounds production making them favorable to use as additive in fish feed.

Previous studies have demonstrated dried algae as a supplementary and protein source substitution in fish diets. Macias-Sancho *et al.* (2014) found that 25% substitution of fishmeal with *Spirulina platensis* promoted the immunological parameters in white leg shrimp, *Litopenaeus vannamei*. Sorensen *et al.* (2016) replaced up to 6% of fishmeal with *Phaeodactylum tricornutum* (a microalga) in diet of Atlantic salmon, *Salmo salar* without detecting any unfavorable effects on nutrient digestibility and growth performance. Other studies have shown that adding Spirulina meal in feed could significantly effect on growth, survival and body carcass of *Litopenaeus schmitti* shrimp (Jaime-Ceballos *et al.*, 2005). Moreover, algae live in an oxygen-rich environment with intense light, both of which result in creation of free radicals; so, different studies have revealed the algae extracts are capable to clear-out free radicals in fish tissues (such as liver or gills) and can be as a natural antioxidant (Natrah *et al.*, 2006; Ganesan *et al.,* 2008; Onofrejova *et al.,* 2010). By catalyzing the conversion of superoxide anion into molecular oxygen and water, superoxide dismutase and catalase are key antioxidant enzymes that were previously have been shown to be present in the fish liver (Aras *et al*., 2009). However, studies evaluating inclusion of algae in modern fish feed have been done just recently.

Microalgae extract (as the second generation of microalgae products) has newly emerged in the market, as it can

be used in a variety of products (Plaza *et al.,* 2008). Studies have shown that algae extract has antimicrobial and antioxidant effects and also used to protect cells against free radicals in human cells (Oh *et al.,* 2011) and promote survival in electric Yellow cichlid (*Labidochromis caeruleus*) (Pezeshk *et al.,* 2018). *S.platensis* is a promising microalgae candidate because of being rich in nutrients. It contains essential amino acids, B group of vitamins, essential fatty acids, antioxidant carotenoids and pigments (Jaime-Ceballos *et al.,* 2005; Goksan *et al.,* 2007; Volkmann *et al.,* 2008). Therefore, using of natural components in Spirulina algae extract, could serve as an alternative in fish diet as a natural substitute versus of synthetic material; moreover, extract of algae and terrestrial plants can use in low amount specially for carnivore fish such as Rainbow trout.

As Rainbow trout is considered globally as an important cold-water species in aquaculture, we studied the effects of methanolic extract of *S. platensis* algae on juvenile Rainbow trout by measuring growth performance, survival, activity of liver antioxidant enzymes and plasma metabolites after stressed animals were fed on different amounts of Spirulina.

Materials and methods

Preparation of algae extract and diets

S. platensis algae powder has been obtained from micro-algae Parsian (Rasht, Iran) and transferred to Sari Science and Technology Park (Mazandaran, Iran). The dried algae (50 g) were crushd with mortar and pestle and the powder was transferred to screw cap glass containers and mixed with chloroform (1:6 w/v) and methanol (90%) (2:1 w/v) (Babakhani *et al.,* 2016). The containers were shaken well and incubated at room temperature for 24 hours in dark. During these 24 hours, containers were shaken for several times in order to make the settled powder into solution. After passing through the filter paper, the solvents were evaporated in a rotary evaporator (TAT-94-1046, Teif Azma Teb, Tehran, Iran) at 35 $^{\circ C}$. The concentrated extract (with approximately 8- 10 % yield) was stored at 4°C in 10 ml of methanol (Kumari *et al.,* 2011). This methanolic extract of Spirulina after removing solvent, was added to 10 ml olive oil and 20 ml lukewarm water to appear a homogeneous mixture. The mixture was sprayed (Boonyapakdee *et al.,* 2014) in different dosages (0, 250, 500, 1000 and 2500 mg per kg of diet) on the Rainbow trout commercial feed (Table 1) and then dried at room temperature. The diets were finally packed and stored at - 20°C until used.

Rearing procedures

A group of 150 Rainbow trout juveniles were obtained from cold-water fish's culture center, Ghezel Sara (Mazandran, Iran) .Fish were fed on Rainbow trout commercial pellet (as the same on in Table 1) for two weeks to get adapted to the experimental diet and environmental conditions. Fish weighted (initial body weight 12.6±0.3 g) and randomly supplied in 15 of 100-

L circular fiberglass tanks (n=10 per tank), in a flow through system, which was constantly aerated. The tanks were cleaned and siphoned to remove debris and dead fish on a daily basis. Temperature, dissolved O_2 and pH-value were kept at 10.6±0.6 °C, 8.5 mg L^{-1} and 7-8, respectively. Five groups of fish were fed manually to apparent satiety with the experimental diets four times a day (Esmaeili and Khara, 2014) (8:00, 10:00, 14:00 and 18:00 h) for eight weeks. Three tanks have been used for each condition.

Samples preparation

At the end of the feeding experiment, the animals were anaesthetized moderately with clove powder (120 mg L^{-1}) (Anderson *et al.,* 1997) and then individually weighed (accuracy of 0.01 g) and lengthen (accuracy of 0.1 cm) in order to determine their final body weight. Three fish form each tank (9 per treatment) has been used for sampling. Blood samples were taken from the caudal vessels with 2 ml heparinized syringe, which was followed by killing the fish by a sharp blow in the head. After 10 minutes of blood centrifugation at 3000 g, 4 °C (K24, Centurion Scientific Centrifuge, Germany), plasma samples were stored at -20 °C for future analyses (Babaei *et al.,* 2017). Body muscle and liver tissue were dissected using clean tools on ice (0 ºC), washed up and immediately frozen at -20 ºC and -80 ºC, respectively. Fish growth performance and survival rate were calculated in order to:

Specific growth rate = ((Ln FBW−Ln IBW)×100/Experiment time (Mohanta *et al.,* 2008)

Condition factor = $(FBW/L^3)*100$ (Denstadli *et al.,* 2006)

Feed conversion Ratio = Dry feed consumed (g)/ (FBW-IBW) (g) (Mohanta *et al.,* 2008)

Survival (%) = (Number of fish in each group remaining in end of experiment/ initial number of fish) × 100 (Hamza *et al.,* 2008)

IBW: Initial body weight

FBW: Final body weight

Chemical composition analysis

Crude protein, lipid and moisture contents of the fish muscles were determined using methods (AOAC, 2005); at the end of experimental period, fish were headed and gutted. Total protein content was measured by Kjeldahl system (230-Hjeltec Analyser; Foss Tecator, Hoganas, Sweden) and total lipid by automatic Soxtec system (2050-FOSS; Sweden). Moisture content was determined by drying at 105 $^{\circ C}$ for 24 h in oven (D-63450; Heraeus, Hanau, Germany) and ash by burning in a muffle furnace (KLI-14; PECO; Shiraz, Iran) at 550 °C for 6 h.

Biochemical analyses of plasma parameters

All biochemical tests were done using clinical diagnostic kits. Plasma lysozyme concentration has been determined with the ZB-LZM96A kit (ZellBio GmbH, Germany) and spectrophotometry (Automatic analyser, Hitachi 902, Japan) at 450 nm (Ellis, 1990).

Plasma glucose concentration has been determined by a kit (Ref. number: 150017, Pars azmoon, Karaj, Iran) based on a colorimetric glucose oxidase –peroxidase reaction and Auto analyzer system (Automatic analyser, Hitachi 902, Japan) at 546 nm. Plasma cortisol concentration has been determined using commercially available ELISA kit (lot num. 36101102, Diaplus Inc., USA) with ELISA reader system (Awarness, stat fax-2100, USA).

Liver antioxidant enzyme extraction

Fish liver was homogenized (1:10, w/v) in 100 mM potassium phosphate buffer (pH 7.4), 100 mM KCl and 1 mM EDTA at 4°C using an electric homogenizer (MM400; Retsch, Germany) for 1.5 min. Homogenates were centrifuged at 10,000 g (Hermle Labortechnik, Germany) for 35 min at 4 °C (Babaei *et al.*, 2017). Supernatants were kept to determine superoxide dismutase (SOD; EC 1.15.1.1) and catalase (CAT; EC 1.11.1.16) activities (Atli and Canli, 2010).

Determination of SOD and CAT activities

CAT was assayed using the ZB-CAT96 kit (ZellBio GmbH, Germany). CAT activity unit was considered as the amount of the sample that catalyzed decomposition of 1 μmol of H_2O_2 into H_2O and O_2 per minute. Absorbance was recorded at 405 nm. SOD was assayed with the ZB-SOD96 kit (ZellBio GmbH, Ulm, Germany). SOD activity unit was considered as the amount of the sample that catalyzed decomposition of 1 μmol of O^{2-} into H_2O_2 and O_2 per minute. Absorbance was recorded at 550 nm.

Total soluble protein has been analyzed by the Bradford method (1976) using bovine serum albumin as a standard curve by reading OD_{546}. Enzyme activities were expressed as specific activity (U mg^{-1} protein).

Air exposure and confinement stress test

At the end of experiment, four fish from each tank (12 per treatment) were randomly selected and kept outside of water for 30 seconds (air exposure stress). Then, they were transferred in small baskets in water for one hour (increasing fish density from 10 g L^{-1} to 100 g L^{-1}) as a confinement stress (Pottinger and Pickering, 1992; Costas *et al.*, 2008). Three and eight hours after the stress, fish were anaesthetized and blood have been collected from fish as mentioned earlier. Blood plasma was stored at -20 ºC for glucose and cortisol measurements.

Statistical analyses

Data were first checked for normality (Kolmogorov–Smirnov test) and homogeneity of variances before any evaluation. Data were analyzed by one-way ANOVA to determine the main effect of different amount of Spirulina extract on growth performance, survival, enzymes activities and plasma metabolites. Statistical significance was set at reliability level of 0.05 and all results were reported as mean values± standard deviation. Software SPSS (version 20) was used for statistical

analyses and Excel (version 2010) for drawing the charts.

Result

Growth performance and body composition

The results of the growth performance and survival rate of Rainbow trout in various treatments are presented in Table 2. After eight weeks, by adding different amounts of *S. platensis* extract to diets, have been induced a significant difference between treatments (p<0.05). Treatments 2 and 5 (T2 and T5) resulted in the highest (84±3.4 g) and lowest (66± 5.3 g) final body weight (p<0.05), respectively. Similar trend was seen with SGR (p>0.05).

The maximum and minimum FCR values were seen in fish diets T1 (control group) and T2, respectively (p<0.05). (However, FCR values in T2 was not significantly lower than T3, T4 and T5) (p>0.05). At the end of experiment, in all treatments fed on diets containing Spirulina extract, the survival rate was higher compared to the control group. However, they were not significantly different form one another. In addition, there is no significant difference between treatments in the condition factor (Table 2). Body composition of Rainbow trout juveniles did not show any significant difference among different treatments at the end of the experiment period (p>0.05) (Table 3).

Plasma metabolites

Neither plasma cortisol nor glucose level (Figs. 1 and 2) were significantly different between the treatments at the end of the feeding period (time 0), yet three hours after entering the confinement stress, plasma cortisol levels was increased in all groups (p>0.05). The plasma cortisol in all treatments decreased after eight hours upon the stress, but there was no significant difference between treatment (p>0.05) except T2 (p<0.05). Eight hours after the stress, the highest level of cortisol has been detected in T3, but no significant difference was found in others.

Plasma glucose decreased eight hours after stress (Fig. 2), while T3 showed the highest level in different times after stress just as it was seen with cortisol amount. Glucose level decreased in T1, T2 and T4, after three and eight hours upon the stress (p<0.05). As shown in Fig. 3, the lowest amount of lysozyme activity was related to T4. However, other treatments were not affected by addition of algae extract in the diets (p>0.05).

CAT and SOD specific activities in liver

The specific activities of SOD and CAT in Rainbow trout liver were evaluated at the end of feeding period (Figs. 4 and 5). The SOD specific activity was not significantly different in T2, T3 and T4 in comparison to the control group (p>0.05). However, it was the highest in T5 (p<0.05). The results did not show any changes in CAT activity between treatments (Fig. 5).

Table 1: Formulation and chemical composition of experimental diets used to feed Rainbow trout juveniles.

Treatment 1*	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Commercial Feed** + 10 ml olive oil + 20 ml lukewarm water per kg of diet	Commercial D + 10 ml olive oil + 20 ml lukewarm water + 250 mg of Spirulina extract per kg of diet	Commercial Feed + 10 ml olive oil + 20 ml lukewarm water + 500 mg of Spirulina extract per kg of diet	Commercial Feed + 10 ml olive oil + 20 ml lukewarm water + 1000 mg of Spirulina extract per kg of diet	Commercial Feed + 10 ml olive oil + 20 ml lukewarm water + 2500 mg of Spirulina extract per kg of diet

*Control treatment

** Mazandaran Animal & Aquatic feed (Manaqua) Co. (Protein: 46%, Fat: 13%, Ash: 7% Moisture: 5%)

Table 2: Growth performance of Rainbow trout juveniles fed on the experimental diets for 56 days.

Growth parameters	Treatments				
	T 1*	T 2	T 3	T 4	T 5
IBW (g)‡	12.5 ± 0.06	12.9 ± 0.31	12.4 ± 0.5	12.8 ± 0.17	12.6 ± 0.35
FBW (g)¶	75 ± 2.3 ^{ab}	84 ± 3.4 ^{a}	73 ± 15.1 ^{ab}	73 ± 4.9 ^{ab}	66 ± 5.3 ^{b}
Length	19.3 ± 0.3 ^{ab}	20 ± 0.3 ^{a}	19.4 ± 0.9 ^{ab}	19.3 ± 0.5 ^{ab}	18.8 ± 0.5 ^{b}
SGR (% day $^{-1}$)◊	2.9 ± 0.1	3.1 ± 0.1	2.9 ± 0.5	2.8 ± 0.2	2.7 ± 0.1
CF ♦	1	1	1 ± 0.1	1	1
FCR (g^{-1})§	1.2 ^{a}	0.9 ^{c}	1.1 ± 0.1 ^{b}	1 ± 0.1 ^{bc}	1 ± 0.1 ^{bc}
Survival (%)†	93 ± 5.8	100	100	97 ± 5.8	97 ± 5.8

* Control group

‡ Initial body weight at the first day of experiment

¶ Final body weight after 8 weeks feeding

◊ Specific growth rate = ((Ln FBW−Ln IBW)×100 /duration (56 days) (Mohanta *et al*., 2008)

♦ Condition factor (CF) = (FBW /Length^3) (Denstadli *et al*., 2006)

§ Feed conversion Ratio=Dry feed consumed (g)/ (FBW-IBW) (g) (Mohanta *et al*., 2008).

†† Survival (%) = (Number of fish in each group remaining in end of experiment/ initial number of fish) × 100 (Hamza *et al*., 2008).

Small letters indicate statistical differences between different groups (p<0.05) (one-way ANOVA). Values are means ± S.D. (n=3; number of tanks per treatment).

Table 3: Chemical composition of juvenile Rainbow trout muscle fed on the experimental diets.

Chemical composition	Treatments				
	T 1*	T 2	T 3	T 4	T 5
Moisture	73.9 ± 0.7	73.2 ± 0.5	74.1 ± 0.1	74.5 ± 0.4	73.4 ± 2.3
Protein	15.9 ± 1.7	16.7 ± 0.7	16.7 ± 1.4	15.68 ± 0.8	16.43 ± 0.4
Fat	7 ± 1.2	7.2 ± 0.5	7.1 ± 1.2	6.52 ± 0.4	7 ± 0.9
Ash	1.7 ± 0.4	1.63 ± 0.08	1.5 ± 0.05	1.45 ± 0.05	1.46 ± 0.3

Small letters indicate statistical differences between different groups (p<0.05) (one-way ANOVA). Values are means ± S.D. (n=3; number of tanks per treatment).

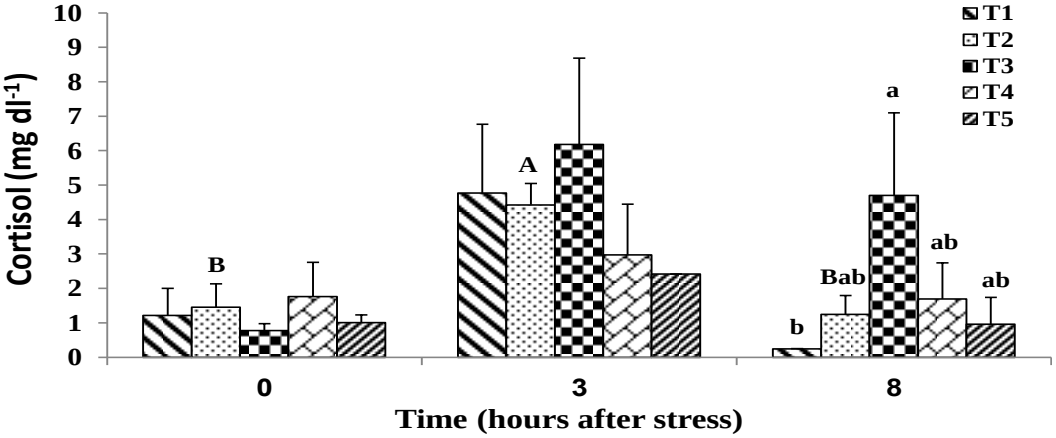

Figure 1: Plasma cortisol level of Rainbow trout fed on diets differing in the amount of Spirulina extract. Capital letters indicate statistical differences between time points after stress within the same group and small letters indicate statistical differences between the groups in a specific time point after stress (p<0.05) (one-way ANOVA). Values are shown as means±SD (n = 3; number of tanks per treatment).

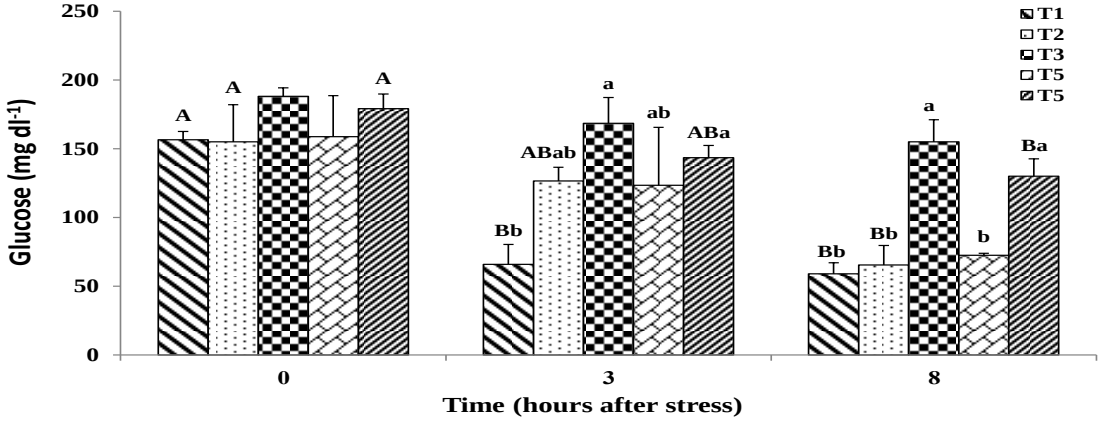

Figure 2: Plasma glucose level of Rainbow trout fed on diets differing in the amount of Spirulina extract. Capital letters indicate statistical differences between time points after stress within the same group and small letters indicate statistical differences between the groups in a specific time point after stress (p<0.05) (one-way ANOVA). Values are shown as means±SD (n=3; number of tanks per treatment).

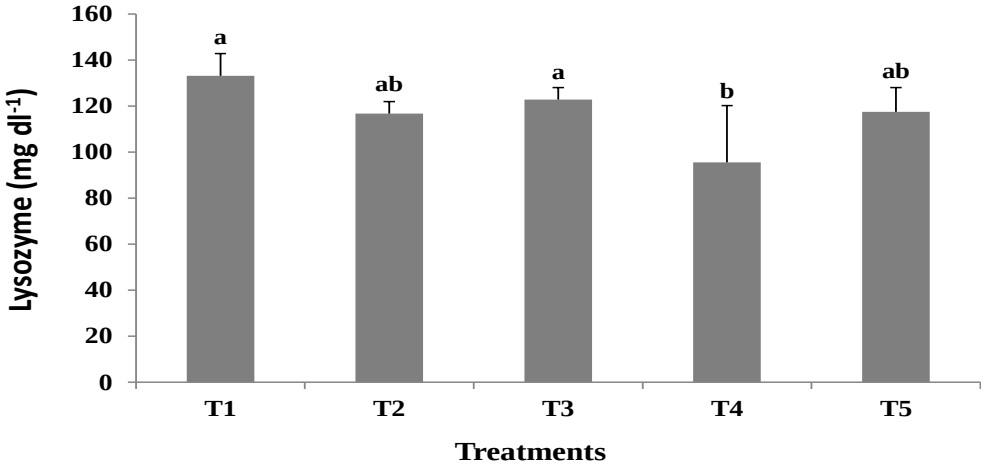

Figure 3: Activity of plasma lysozyme in Rainbow trout fed on diets differing in the amount of Spirulina extract. Different values of enzyme activity (means±SD, N=3 tanks) with different superscript letters are statistically significant (p<0.05).

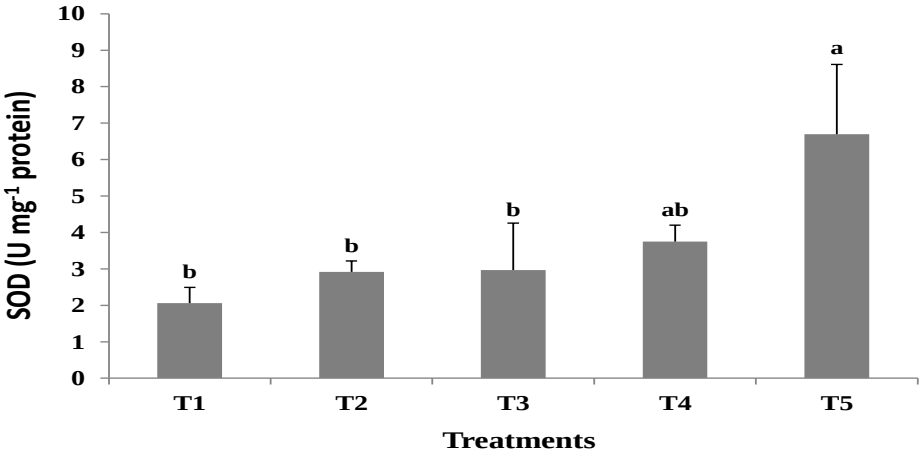

Figure 4: Specific activity (U mg protein^{-1}) of SOD in Rainbow trout liver after 8 weeks of feeding. Different values of enzyme activity (means±SD, N=3 tanks) with different superscript letters are statistically significant (p<0.05).

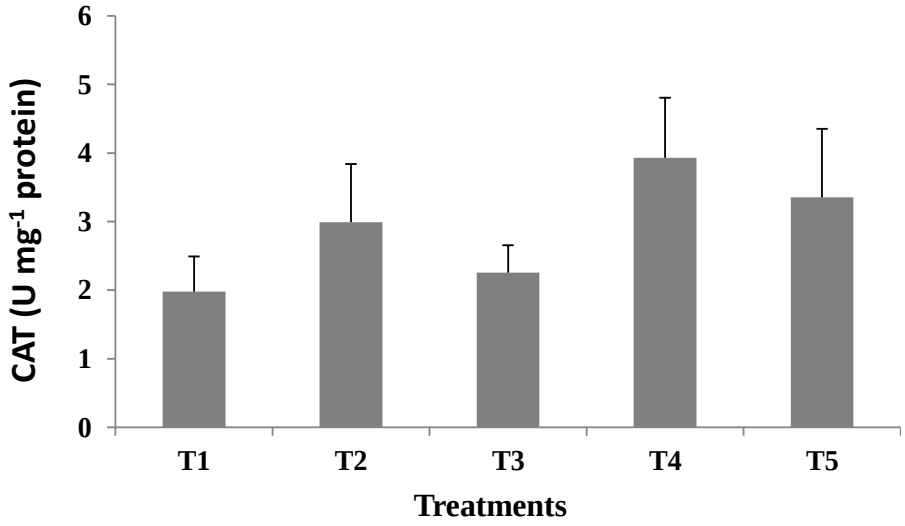

Figure 5: Specific activity (U mg protein^{-1}) of CAT in Rainbow trout liver after 8 weeks of feeding. Means±SD, N= 3 tanks.

Discussion

The interest in using commercially grown microalgae for biofuels, feed and pharmaceuticals has been growing (Kiron *et al.,* 2012). Although many studies have investigated supplementation of fish diet with Spirulina powder (Olvera-Novoa *et al.,* 1998; Nandeesha *et al.,* 2001; Palmegiano *et al.,* 2008), using microalgae extract as an immune and antioxidant stimulator or feed attractant is left to be studied. In this study, adding methanolic extract of *S. platensis* in Rainbow trout diet led to significant effects on growth and SOD activity.

In the present study, changes in growth indices among different treatments proved that adding algae extract has significant effects on fish growth performance. Juvenile Rainbow trout gained weight 6 times more than their initial one at the end of the experiment when fed on Spirulina extract. This is in agreement with other

studies using Spirulina as feed supplement. Arney *et al.* (2015) reported higher growth rate in shells of Pacific geoduck clam (*Panopea generosa*) due to inclusion of microalgae Spirulina extract in diet. Our results showed that addition of the Spirulina extract 250 mg kg^{-1} of Rainbow trout diet resulted in increased final weight and survival, and significantly decreased FCR value compared to control group. Previous studies have also shown that addition of small amount of Spirulina (0.05 % of the diet) could work as a feed attractant for *L. vannamei* juveniles (Silva-Neto *et al.*, 2012).

No significant difference was detected between treatments in the muscle proximate analysis in this study. On the contrary, Choi *et al.* (2015) showed increased fat content of Olive flounder *Paralichthys olivaceus* fed on Red algae *Pyropia yezoensis* extract. Abdulrahman (2014) also observed increased crude protein in Common carp by addition of %15 *Spirulina* sP. algae powder. However, use of *Scenedesmus almeriensis* microalgae in the diet of sea bream (*Sparus aurata*) had no significant effect on muscle composition (Vizcaino *et al.*, 2014). Considering the aforementioned studies, no significant difference in Rainbow trout body composition might be attributed to the use of algae dried biomass in other studies, which might be more effective than algae extract on body muscle.

Our results showed that using methanolic extract of Spirulina in the diet did not have any significant effect on lysozyme concentration in Rainbow trout plasma and decreased when 1000 mg extract was added per kg of feed. Warm water extract of microalgae *Gracilaria tenuistipitata* in diet of white shrimp (*L. vannamei*) induced higher lysozyme activity in parallel with the amount of extract used (Siriustananum *et al.*, 2011). Moreover, Watanuki *et al.* (2006) and Saligheh Zadeh *et al.* (2015) reported similar results of immune system improvement from Spirulina powder replacement in the diet of Common carp and Benny fish (*Mesopotamichthys sharpeyi*), respectively. In a different study, immersion of white shrimp in seawater containing Spirulina algae extract induced higher lysozyme activity directly with increasing the extract concentration (Tayag *et al.*, 2010). Unlike these studies, we did not find any evidence supporting positive effect of methanolic algae extract on the plasma level of lysozyme, which can be either due to using concentration data instead of lysozyme activity and due to using the aqueous extract of algae by those researchers.

Cortisol and glucose in fish stress studies are measured as reliable indicators of the stress responses (Martínez-Porchas *et al.*, 2009). According to this study, addition of algae extract in the diet of affected the stress response of Rainbow trout. Three hours after the onset of the confinement stress, the level of cortisol was elevated in all treatments and dropped down eight hours after the stress. Consistent with this study, plasma glucose level in juvenile Cobia (*Rachycentron*

canadum), which was fed on Spirulina algae, increased after the stress by immersion in freshwater (Sapurta *et al.*, 2016). In addition, Yeganeh *et al.* (2015) reported that using Spirulina powder in Rainbow trout diet led to significantly reduced cortisol and glucose levels in accordance with decreasing amount of the algae powder. A similar observation was reported about plasma glucose level of Rainbow trout when *Haematococcus pluvialis* has been used as an additive (Sheikhzade *et al.*, 2012). Therefore, based on the results, it can be suggested that adding 250 mg/ kg of Spirulina extract to the diet can reduce stress in Rainbow trout juvenile especially for handling stress.

Antioxidant and DPPH radical scavenging activity have been well known in numerous micro- and macro-algae (Kelman *et al.*, 2012). For instance, ethanol extracts of *Callophyllis japonica* activated cellular antioxidant enzymes (Kang *et al.*, 2005). Different studies have been shown that the extracts from many seaweeds and microalgae are capable of eliminating free radicals in fish tissues and can be used as natural sources of antioxidants (Natrah *et al.*, 2006; Ganesan *et al.* 2008; Onofrejova *et al.*, 2010). When 0.5, 1, 2 g kg^{-1} hot water extract of *Gracilaria tenuistipitata* have been used in the feed of white shrimp *Litopenaeus vannamei*, the activity of SOD showed direct relation with the dosage of extract in feed and period of feeding (Siriustananum *et al.*, 2011). The SOD activity in white shrimp, which was injected with 4 or 6 μg g^{-1} aqueous extract of *Gracilaria tenuistipitata* algae, was increased (Hou and Chen, 2005). In contrast, different amounts of Spirulina extract did not affect antioxidant defense in Rainbow trout liver in this study. Because adding high amount of the algae extract (2500 mg kg^{-1}) induced higher SOD activity, therefore it can suggest lower amount (below 500- 1000 mg kg^{-1} diet) can be used in Rainbow trout diet.

In general, our findings showed that using 250 mg of *S. platensis* extract resulted in improving growth performance, survival rate and FCR in juvenile Rainbow trout. In addition, 250 mg kg^{-1} methanolic extract of *S. platensis* can be considered as an immune booster factor in Rainbow trout, especially during handling and confinement stress.

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