The effect of feeding with hydroalcoholic extract of *Spirulina platensis* on the growth and immune responses of Rainbow trout (*Oncorhynchus mykiss*) fry

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Abstract

Rainbow trout (*Oncorhynchus mykiss*) is the most economically important farmed species in Iran, whose intensive culture is associated with various stresses and makes the fish susceptible to various diseases. Using natural immune stimulants is one of the most effective ways to boost immunity and prevent diseases in fish. *Spirulina platensis* contains protein, vitamins, minerals, essential amino acids, fatty acids and antioxidant pigments such as beta-carotenoid and is considered as a growth stimulant, probiotic and immune system booster in aquatic animals. The present study was conducted in order to investigate the effect of hydroalcoholic extract of *spirulina platensis* algae on growth and immune responses of rainbow trout (*O. mykiss*) fry. A completely randomized experimental design was developed with five test groups including commercial diet as control along with 2, 4, 6 and 8% hydroalcoholic extract of *spirulina* algae each containing three replicates. two thousand and four hundred rainbow trout with average initial weight of 20±0.5 g were assigned to 20 experimental rectangular polyethylene pools (v= 0.3m^3). the adaptation and expriment periods were 15 and 60 days, respectively. Biometry performed once every 20 days. Rainbow trout fed by 8% *S. platensis* (treatment 5) showed a significant difference in percentage of the condition factor (1.16±0.01), Growth Rate (0.41±0.02), Specific Growth Rate (0.38±0.015), Lysozyme Activity (2.77±0.12 mgL^{-1}) and the survival to oxygen depletion (62.96±1.85), temperature (57.40±4.89) and salinity stresses (74.07±1.85) with the other groups (p<0.05). Adding 8% *S. platensis* hydroalcoholic extract as a food supplement was found to be improved the growth, and immune responses performance in rainbow trout fry.

Keywords: *Spirulina platensis*, rainbow trout, growth index, immune response

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Introduction

Rainbow trout is one of the main important economic fishes grown in different parts of the world including Iran. This species has a good global market, and according to the statistics published by FAO in 2016, the total production of rainbow trout was 814000 tons (Sheikhzadeh *et al*., 2019). The amount of cold-water fish farming in the country in 2015 was 140,244 tons, which increased to 190,287 tons in 2020, and this figure in West Azarbaijan province, which is one of the main centers of cold-water fish farming in the country, changed as 8,948 tons in 2015 to 8,514 tons in 2020 (Iranian Fisheries Statistical Yearbook, 2021). Statistics for 2020 compared to 2015 shows a declining trend in the culture of cold-water fish in West Azerbaijan province, which may be due to various reasons, such as the high costs of keeping and producing fry or the increase in the loss of fry due to the reducing the quality of the diet. In case of using alternative food sources such as *spirulina* algae (Grosshagauer *et al*., 2020), it is possible to increase the production and survival of fry by modifying the feeding methods, immune system, and preservation level, and as a result, the final production efficiency can be increased. The effects of *S. platensis* as an immune stimulant and immune system enhancer have been reported in various aquatic animals (Hu, 2004; Phromshthong and Pipattanawattanakul, 2005; Watanuki *et al*., 2006; Promya and Chitmanat, 2011; Sheikhzadeh *et al*., 2019). *Spirulina* is a microscopic multicellular and blue-green flamentous algae belongs to the class of Cyanophyta algae or photosynthetic bacteria. Its color is blue-green and it grows in carbon-rich lakes (Grosshogauer *et al*., 2020). *Spirulina* algae are rich in various vitamins and proteins that can boost the immune system of the animals that feed on them and also increase the ability to absorb and use food in them. Using it in the diet of fish larvae, improves meat color or body appearance, growth performance and reduces losses in fish larvae (Promya and Chitmanat, 2011; Abdel-Tawwab *et al*., 2021). *S. platensis* is widely used to improve nutrient composition and physiological response to stress in many fish species (Takeuchi *et al*., 2002: Van vo *et al*., 2020). It also has a high protein reserve (60-70% of dry weight), vitamins, especially vitamin B12, an approciate amino acid, essential fatty acid profile, and a wide range of pigments, especially phycocyanin (Teimouri *et al*., 2013). According to Velasquez *et al*. (2016), *spirulina*, as a key component in the natural diet of tilapia fish, can replace up to 75% of the protein in the diet of young Nile tilapia without adversely affecting growth or blood chemistry. High amounts of it in the diet have had similar effects on carp. However, *spirulina* in excessive amounts hampers the growth rate of silvercarp and causes pro-oxidant activity in rabbits and chickens. The effect of *spirulina* on the growth performance of fish appears to be

particularly species related since the feeding habits of different organisms significantly influence the ability to digest, store and absorb different nutrients (Raji *et al*., 2020). In a study by Nasrollehzadeh *et al*. (2013), the use of *spirulina* powders up to 2% had a positive effect on the survival of juvenile *Rutilus frisii kutum* but survival rate decreased at 5% treatment. Yousefi *et al*. (2014) reported that the oral intake of *spirulina* in carp increases total protein and some components of serum proteins, including gamma globin. The studies by Biranvand *et al*. (2014) showed that the use of *spirulina* algae in the diet of zebrafish (*Danio rerio*) increased the body weight and specific growth rate of this species, but increased the amount of *spirulina* algae powder in the diet of zebrafish during the rearing period of 60 days did not affect the feed conversion rate. Considering that there was no significant difference between the 1 and 1.5% treatments in terms of growth indices, the 1% *spirulina* algae powder treatment was the best treatment for zebrafish growth. The studies by Salighezadeh *et al*. (2014) showed that the addition of *spirulina* supplements in the diet of benni fish fingerling at the level of 10% improves the final weight, weight gain, specific growth rate, obesity coefficient, and carcass protein content of this species. According to the research of El-daim *et al*. (2021), it is recommended to add 1.5 g per kilogram of *spirulina* algae powder to the diet of gray mullet fish to improve growth indicators, nutrition, and carcass quality and to increase the poly chain fatty acids of this fish. The study conducted by Sodagar *et al*. (2015) showed that the treatments that were fed with a diet containing *spirulina* algae had weight gain (p<0.05). The effect of feeding with different amounts of algae on length gain was not significant (p<0.05). The effect of feeding with different amounts of algae on coloration indices such as lightness index and degree of coloration was also significant (p<0.05). The present research showed that the use of *spirulina* algae in the diet has beneficial effects on the growth indices and some coloration indices of *Pseudotropheus demasoni*. Taghavi Takyar *et al*. (2016) announced that regarding the antioxidant power of the two algae studied, *Chlorella vulgaris* and *S. platensis*, and the side effects caused by the use of synthetic antioxidants (carcinogenic), they suggested that the algae extracts (after conducting additional tests) can be used as a replacement for traditional artificial antioxidants in all types of food. The results of the studies by Ansari Fard *et al*. (2017) showed that adding *spirulina* to the diet increases the percentage of hematocrit and hemoglobin concentration, the white blood cell count, and the red blood cell count of fish. For example, the inclusion of 10% *spirulina* in the diet has significant positive effects on stimulating the immune system of koi fish. According to the studies by Biabani Esrami *et al*. (2016), in dwarf

gourami fish larvae (*Trichogaster lalius*), survival, total carotenoid, and total length increased significantly with the addition of *spirulina* powder. In general, the treatment of 3% algae powders due to the improvement of growth indicators and total carotenoids in the pre-reproductive stage and the treatment of 10% for the larval period is introduced as the best treatment in this study. Based on the research of Salighezadeh *et al*. (2014), 10% *spirulina* supplementation increases complements and lysozyme safety indicators in benni fish. Based on the results of research by Biddy *et al*. (2018), it is suggested that 4% *of spirulina* food grains are suggested to improve growth, nutrition, hematology, and immunological indicators of grass-carp. The study by Sadeghi Goghri *et al*. (2019) shows that the combined diet of lactoferrin and *spirulina* algae powder had a positive effect on some growth and immunity indicators in female zebrafish. Several studies have been conducted to examine the effect of beneficial algae on the growth performance and safety of aquatic animals. Sirakov *et al*. (2012) studied the effect of *S. platensis* algae on the growth performance and carcass parameters of rainbow trout. In this study, the treatments included a 10% algae diet containing and a no-algae diet. The increase in growth and average daily growth in fish fed with 10% algae was higher than in the group fed with a diet without algae, but there was no significant difference. Fish fed with algae showed a significant difference in consumption performance compared to the control group. Promya and Chitmanat (2011) concluded that when *spirulina* algae were used in the diet of Nile tilapia averaging 30 g, reproductive and survival rates increased. Studies by Amer (2016) showed that supplementation with *S. platensis* increases the protective capacity of antioxidants. It also affects some parameters of innate and humoral immunity and does not hurt fish growth. Based on research by Teimouri *et al*. (2019) including 10% *S. platensis* in the diet of rainbow trout can reduce oxidative stress. Therefore, this alga can be used as a potential antioxidant for fish culture. The results of the studies by Mohammadiazaram *et al*. (2020) showed that *spirulina* algae powder at a level of 55 g per kilogram of feed can be used as a convenient natural feed additive to improve the performance of Oscar fish. This research was conducted to investigate the effect of *spirulina* algae extract on enhancing the growth and immune response of rainbow trout fry.

Materials and methods

Animal

2400 rainbow trout fry with average weights of 20±0.5 g were prepared from Maulai fish farm located in Balanj village in Urmia city. Health certificate of fish obtained from the General Veterinary Department of West Azarbaijan province. The fish were kept in 300-liter rectangular tanks for 2 weeks before starting the research to adapt to the new conditions and to

ensure their health. During this trial, the fry were fed commercial concentrated food (Gilak Daneh Navid Factory, Iran) three times a day and their mortality rate were recorded.

Experimental design

A completely randomized experimental design was developed with five test groups including commercial diet as control along with 2, 4, 6 and 8% hydroalcoholic extract of *spirulina* algae each containing three replicates. two thousand and four hundred rainbow trout with average initial weight of 20±0.5 g were assigned to 20 experimental rectangular polyethylene pools (v=0.3m^3). The research duration was 60 days, and biometry was performed on 20% of the fish once every 20 days.

Preparation of hydroalcoholic extract

First, 50 g of dried algae powder was weighed and transferred to an Erlenmeyer flask. It was mixed separately with 500 ml of 96% ethanol alcohol (Merck, Germany). The flasks were shaken and kept in the dark on a stirring magnet for 24 hours. In this case, Erlen's head was covered with parafilm to keep the material from evaporating. After this step, the upper Erlen liquid was poured into the Falcon tube and the lower liquid was discarded. After centrifugation at a speed of 4000 rpm for ten minutes, the upper liquid was passed through Whatman filter paper (42 microns) and then the filtered liquid was placed in the spinner device at a temperature of 45^{o}C to concentrate it. Finally, it was dried by placing it in an oven at a temperature of 55^{o}C. After this step, the sediment was poured into the plate and transferred to the incubator. After the extract was completely dried, it was used in the research (Sharifi Asal and Rumiani, 2022).

Preparation of the Rainbow trout diet

The amount of diet was calculated according to the daily feed determination table and the amount of each meal according to the water temperature (Sarsangi Aliabadi, 2018 and FAO, 2018). The different levels of extract were sprayed and was used up to 15 minutes after adding to fish feed. The feeding frequency was four times in a day (FAO, 2018). To calculate the daily diet, the number of mortality of each treatment was substracted from the total biomass. Before feeding, the number of mortality was collected and recorded early morning.

Growth performance

To measure the growth indices, fish sampling was performed on the days 20, 40, and 60, so that 10 fish were randomly sampled from each tank and their weight and length were measured. After the trial, survival rate, specific growth rate, food conversion ratio, condition factor, moratality, percentage of body weight gain and average daily growth were calculated according to the following equations (Nekuiefard *et al*., 2017):

Survival rate = number of healthy fish at end of experiment.total fish on the first day x 100

Growth rate = (End weight - start weight) ÷ start weight × 100

Specific growth rate = (natural log final weight - natural log initial weight) . length of rearing time (days) 100

Food conversion ratio = given amount of dry food (g). (final weight (g) – starting weight (g)) 100

Condition factor = (final weight.total length in view 3) 100

Percentage of body weight gain = (Final weight (g) – begining weight (g)) . Begining weight) 100

Mean Daily Growth = (final weight _ initial weight) . (final weight × incubation length)

Measuring the level of immunity

Five fish fries randomly selected from each replicate and placed in zippered plastic bags. To measure the activity of the lysozymeso that fish mucus collected on days 0 and 60 . The samples were gently shaken for 1 min to stimulate mucus secretion. Secreted mucus in plastic bags was transfer to 20 ml-Falcon tubes close the ice, and four times the volume of the collected mucus in 10 mM phosphate-buffered saline (PBS)(5.5) added. 7 pH=contains 115 mM micro sodium) was added and centrifuged at 15000 g for 30 minutes at 4^{o}C. Finally, the upper phase was slowly separated and transferred to clean falcon tubes and used to measure the parameters mentioned (Hosienifar *et al*., 2014).

Lysozyme Activity

Lysozyme activity was measured based on the turbidity method presented by Shugar (2000). For this purpose, 50 µL of mucus were mixed with 4M sodium phosphate buffer pH=6.5, transferred to microplates, and incubated at 30°C for five minutes. Then, 50 µL of *Micrococcus luteus* bacteria (0.3 mg/ml bacteria in 40 mM sodium phosphate buffer, pH=5.6) was added and its light absorption was carried out at 30^{o}C for 5 minutes. The degree read at a wavelength of 450 nm (Hosseini *et al*., 2017).

Environmental stress study

At the end of the experiment, 24 fish divided from each treat group (six fish per each treatment and replicate) were randomly selected and subjected to the stress of oxygen deplition, elevated temperature and salinity. To avoid errors in the conclusion, the sampled fish were subjected to only one stress and to one type of stress.

Oxygen depletion stress

The 18 fish of each treatment were placed in a cage for 10 min to measure the anoxia stress in a 20-liter polyethylene tank, and then put back into running water at the temperature of the trial (15^{o}C). The mortality was measured during the stress test and the resistance of fish to anoxia as oxygen

depletion test was measured after 24 hours (Takami *et al*., 2005).

Thermal stress

The 18 fish of each treatment were subjected to a heat stress at 25°C (10°C higher than the temperature of the rearing period) for three h and then placed back into running water at the temperature of the rearing period. Fish resistance to different heat stress treatments was measured by counting fish mortality during the stress period and then up to 24 hours (Asadi *et al*., 2010).

Salinity stress test

The 18 fish from each treatment were placed in 35% salinity water for three hours, after which time, they were placed back in normal running water. Similar to the previous experiments, the number of fish mortality was recorded during the salinity stress period and up to 12 hours thereafter (Niroomand *et al*., 2011).

Statistical analysis

Statistical analysis was performed using SPSS version 18 software and each treatment was tested in triplicate. The normality of the data was checked with the Kolmogorov-Smirnov test and the homogeneity of the variance of the data was checked with the Leuven test. Duncan's test and analysis of variance were used to compare means (Sarsangi Aliabadi, 2018). The allowable error for rejecting H was 0.5%. The diagram was drawn using Medcalc software (version 13) (Schoonjans, 2008).

Results

Condition Factor (CF)

The comparison (mean±standard error) of the condition factor (CF) on different study days and groups is given in Table 1. By examination the results obtained from the comparison (mean±standard error) of the condition factor (CF) in the examined fish in different test groups on the 20th, 40th and 60th days of the study, there was no significant difference between the experimental groups in this index (p> 0.05) (Table 1).

Table 1: Comparison (mean±standard error) of condition factor (CF) on different study days and groups.

Biometry day/test group	Control	2%	4%	6%	8%
1	1.06±0.01	1.06±0.01	1.06±0.01	1.06±0.01	1.06±0.01
20	1.11±0.01	1.17±0.01	1.14±0.01	1.13±0.01	1.13±0.01
40	1.19±0.01	1.19±0.01	1.14±0.01	1.14±0.01	1.15±0.01
60	1.15±0.01	1.15±0.01	1.16±0.01	1.16±0.01	1.16±0.01

Growth Rate

The comparison (mean ± standard error) of growth rate (GR) on different days and groups of the study is given in Table 2. In the 20th day of biometry, the comparison of the growth rate (mean±standard error) showed a significant difference between the experimental groups of 6% and 8% among themseleves and between these

two experimental groups and other groups ($p< 0.05$).) so that the control groups and two percent showed no significant differences from each other ($p> 0.05$). In the 40th day biometry, the comparison of the mean growth rate between the test groups showed no significant difference ($p> 0.05$). Thus, the control groups, 2, 6 and 8% had no significant differences among themeselves ($p> 0.05$). The results obtained in comparing the growth rate index at day 60 showed a significant difference between the control group and 2 groups with 4, 6 and 8% ($p< 0.05$). The experimental group showed a significant difference of eight percent to all the experimental groups ($p< 0.05$) (Table 2).

Table 2: Comparison (mean± standard error) of growth rate (GR) on different study days and groups.

Biometry day/test group	Control	2%	4%	6%	8%
20	0.11±0.00	0.12±0.06	0.14±0.007	0.17±0.008	0.20±0.009
40	0.20±0.009	0.19±0.009	0.19±0.009	0.18±0.008	0.19±0.009
60	0.28±0.013	0.31±0.015	0.34±0.016	0.37±0.018	0.41±0.002

Special Growth Rate

The comparison (mean±standard error) of the specific growth rate (SGR) on different study days and groups is given in Table 3. In the biometry at day 20, comparison of the mean specific growth rate index (SGR) showed a significant difference of 6% and 8% between the experimental groups and other groups ($p< 0.05$). In the biometrics at day 40, a significant difference was observed comparing the specific growth rate between the 6% and 8% experimental groups as well as the 4% group with other experimental groups ($p< 0.05$), such that the control group and 2% and 6 and 8% were not significantly different from each other ($p> 0.05$). The comparison of the mean specific growth rate at day 60 showed a significant difference in the experimental groups of 6% and 8% with other groups ($p< 0.05$), so that the control group was different from all the groups. The results of the test had a significant difference ($p< 0.05$). In the biometrics of the 60th day ($p> 0.05$). The test groups and eight percent and two and four percent showed no significant differences from one another (Table 3).

Table 3: Comparison (mean± standard error) of specific growth rate (SGR) on different days and groups in the study.

Biometry day/test group	Control	2%	4%	6%	8%
20	0.37±0.014	0.37±0.014	0.42±0.016	0.49±0.019	0.54±0.021
40	0.38±0.015	0.40±0.016	0.14±0.007	0.29±0.01	0.20±0.11
60	0.30±0.012	0.32±0.012	0.35±0.014	0.37±0.014	0.38±0.015

Lysozyme activity

The comparison (mean±standard error) of lysozyme activity (mg/liter) of test groups using *spirulina* algae on rainbow trout is given in Table 4. Comparison (mean±standard error) of the lysozyme activity index (mg/liter) of the control group, except for the 2% test group which showed no significant difference (p<0.05), showed a significant difference with the other test groups (p<0.05). The four and six percent test group showed a significant difference with all the groups (p<0.05), but they did not have a significant difference with each other (p<0.05). Comparison (mean±standard error) of lysozyme activity index (mg/liter) in the eight percent test group with 2.77±0.122 mg/liter using *spirulina* algae, had a significant difference in increased activity compared to other groups (p<0.05) (Table 4).

Survival rate

The comparison of mortality and survival (percent) in the test groups during the study is given in Table 5. Comparing of the percentage of mortality and survival in different experimental groups with *spirulina* algae showed no significant difference (p<0.05). According to the obtained results, the use of eight percent of *spirulina* algae diet had the least mortality (0.75%) and the highest survival rate (99.25%) compared to other groups (Table 5).

Table 4: Comparison (mean± standard error) of lysozyme activity (mg/liter) of test groups using *spirulina* algae in rainbow trout.

Test group	Index (mg.liter)	mean ± SE
Control	*Lysozyme activity*	1.06±0.024
2%	*Lysozyme activity*	1.17±0.43
4%	*Lysozyme activity*	1.63±0.097
6%	*Lysozyme activity*	1.87±0.024
8%	*Lysozyme activity*	2.27±0.122

Table 5: Comparison (percent) of casualties and survival in test groups during the study.

Test group	casualties (percentage)	Survival (percentage)
Control	1.5	98.5
2%	1.25	98.75
4%	1.25	98.75
6%	1	99
8%	0.75	99.25

Stress tests

The comparison (mean ± standard error) of the survival rate at different stresses at the end of the period in the studied treatments is given in Table 6. The comparison (mean±standard error) of the survival rate of the stress test in different experimental groups showed the highest survival (percent) for oxygen, thermal and salinity stresses, 62.96±1.85, 57.40±4.89 and 74.07±1.85, respectively in the test group, 8% usage of *spirulina* algae. The lowest survival percentage for oxygen, thermal and salinity stresses was obtained in the control group,

64.81±1.85, 61.11±3.20, and 79.62±1.85, respectively (Table 6).

Table 6: Comparison (mean± standard error) of the survival rate at different stresses at the end of the period in the studied treatments.

Test stress/group	Control	2%	4%	6%	8%
Oxygen tension	64.81±1.85	64.96±1.85	64.81±4.89	68.51±1.85	62.96±1.85
Heat tension	61.11±3.20	61.11±5.55	64.81±4.89	70.37±6.67	57.40±4.89
Salinity stress	79.62±1.85	81.48±1.85	87.03±1.85	90.74±3.70	74.07±1.85

Growth indices

The comparison (mean ± standard error) of growth indices including weight and length of fish in different test groups on days 1, 20, 40 and 60 of the study was measured and subjected to statistical analysis. The comparison (mean± standard error) of the growth indices of rainbow trout in biometric times in different test groups is given in Table 7**.**

In the day-60 biometrics, comparison of averages of length and weight showed a significant difference between the control group and other results obtained, indicating a significant difference in length and weight in the control group and two percent with other treatments (p<0.05). The maximum weight and total length at the time of the final biometry (day 60) related to the test group with the 8% *spirulina* algae diet (test group 5) were 12.03±0.03 and 19.40±0.05, respectively and the lowest of these indicators at the same time was related to the control group with the average total weight of 11.13±0.03 and total length of 15.30±0.05. Comparison of mean values at the day 20 showed that the two percent treatment and the control had no significant difference (p<0.05), but there was a significant difference with the other treatments, the same comparison showed a significant difference in the intergroup treatments 5-3 (p<0.05). Comparing of the longitudinal mean of the treatments showed no significant difference between the 2% and 4% control test groups, but a significant difference was observed in the 6% and 8% groups (p<0.05).In the 40th day biometrics, the comparison of the average weight in different test groups showed a significant difference between the control groups and two percent with other test groups (p<0.05).Comparison of mean length in biometrics at day 40 showed a significant difference between control groups and 2% with other test groups (p<0.05).

Table 7: Comparison (mean± standard error) of growth indices of rainbow trout in biometric times in different test groups.

	Indicator	Control	2%	4%	6%	8%
First day	Total weight (g)	3.10±0.05	3.10±0.05	3.10±0.05	3.10±0.05	3.10±0.05
	Total length (cm)	6.70±0.05	6.70±0.05	6.70±0.05	6.70±0.05	6.70±0.05
20th day	Total weight (g)	5.53±1.17	5.80±0.05	6.13±0.08	6.76±0.08	7.23±0.03
	Total length (cm)	8.03±0.06	8.00±0.05	8.20±0.05	8.53±0.06	8.73±0.03
40th day	Total weight (g)	9.46±0.13	9.66±0.03	9.76±0.03	9.80±0.05	9.80±0.05
	Total length (cm)	9.60±0.05	9.80±0.05	10.10±0.05	10.40±0.01	11.10±0.05
60th day	Total weight (g)	11.13±0.03	11.30±0.00	11.53±0.03	11.73±0.03	12.03±0.03
	Total length (cm)	15.30±0.05	16.16±0.03	17.13±0.03	18.06±0.03	19.40±0.05

Discussion

Many species of algae, plants and plant extracts (aqueous, methanolic, ethanolic) are currently being used successfully in the aquaculture industry and have a positive effect on fish growth, antioxidant status, immunity and resistance to waterborne pathogens. Extracts of various plants can improve the food conversion rate, shorten culture time for market supply and reduce breeding time costs (Javad *et al*., 2009). The commercial blue-green filamentous *spirulina* algae have the potential to be used as a probiotic growth stimulator and immune system booster in aquatic animals that are abundantly produced as it does not require a special environment to growth and can grow outdoors. Cultivated without the presence of pollutants or other algae and starters. Dried *spirulina* powder is high in protein (up to 55-70% dry weight). It also contains a large amount of gamma-linolenic acid (GLA), polysaccharides, phycobili proteins, carotenoids, vitamins (especially B12) and pigments such as carotenoids and minerals. Some studies also reported the stimulating effects of *spirulina* on the immune system in several species of fish. Besides carotenoids, *spirulina* also contains other pigments called phycocyanobilin, including phycocyanin, allophycocyanin, and phycoerythrin, which are responsible for antioxidant activities in the body. One of the main problems of fish reproduction and culture is the large loss of fry, which according to most experts, the nutrition and health problem, especially at the beginning of external feeding and the fingerlings in this regard, will lead to the weakening and reduction of the fry quality and as a result, the mortalities increase. Raising fish fry is all about providing, high quality food that is easily accepted and digested by the fish fry. Improving nutritional and physiological conditions can be considered an effective step to increase rainbow trout production. In this study, the growth indices and immune responses of rainbow trout fry fed with 2, 4, 6 and 8% hydroalcoholic extract of *spirulina* algae were examined. The results showed that the experimental group with 8% *spirulina* algae had the lowest mortality (0.75%) and the

highest survival rate (99.25%) compared to other groups. In the oxygen, thermal and salinity stress tests, the highest survival percentage was observed in the experimental group of eight percent algae. The lysozyme activity index in the test group of eight percent algae showed increased activity compared to other test groups. In biometrics, the maximum length and weight of the test group contained eight percent of *spirulina* algae. Beidi *et al*. (2018) suggested four percent *spirulina* dietary grains to improve the growth, nutritional, hematology and immunology indicators of grass carp. The results of the studies by Ansari Fard *et al*. (2016) showed that the inclusion of 10% *spirulina* in the diet has significant positive effects on stimulating the immune system of koi fish. Promya and Chitmanat (2011) reported that catfish (*Clarias gariepinus*) fed with a diet containing 5% *spirulina* algae showed the highest level of immunity compared to other treatments, which is consistent with the results of the present study. In the growth rate index in the present study, the treatment group with 8% *spirulina* algae had the highest value at day 60 and showed a significant difference from all the test groups. There was no significant difference between the specific growth rate of 6 and 8 percent of the experimental groups and the 2 and 4 percent in biometrics at day 60 ($p> 0.05$). Thereafter, the group with two percent algae, which had a higher value than the control group and had a statistically significant difference from this group, was the appropriate treatment for the specific growth rate from an economic point of view. The positive performance on growth indicators shows that adding *spirulina* to the diet can increase the efficiency of food intake by increasing the intestinal bacterial colony. James *et al*. (2009) suggested that *spirulina* by increasing the gut flora of the fish, breaks down the indigestible food and removes more nutrients from the diet. In addition, *spirulina* can stimulate the secretion of enzymes that ensure fats are consumed for fish growth instead of being stored. In the study by Olvera-Novoa *et al*. (1998) and Dernekbasi *et al*. (2010) found that replacing fishmeal with *spirulina* did not reduce growth in tilapia and guppy by up to 40% level, respectively. It has also been reported that *spirulina* at lower concentrations can improve growth performance. On the other hand, Olvera-Novoa *et al*. (2008) found that higher concemtrations of *spirulina* algae (60, 80 and 100%) resulted in a decrease in growth indices in tilapia fish (*Oreochromis mossambicus*). Mustafa *et al*. (2007) and Guroy *et al*. (2012) suggested values of five and ten percent for sea bass and yellowtail cichlids, respectively, to improve growth factors. Kermani *et al*. (2020) found that adding 250 mg/kg of *spirulina* extract to the diet of rainbow trout diet can improve growth performance and stress conditions. Meshkat Roohani *et al*. (2020) reported that Caspian brown trout fry fed with diets containing six and eight percent *spirulina* algae

powder showed higher weight gain and specific growth rate compared to the control group. Also, in the research of Meshkat Roohani *et al*. (2020), increased lysozyme enzyme activity by 6% increasing dietry *spirulina* levels and then decreased by 8% with treatment, which is inconsistent with the results of the current research on lysosomal activity. Byeon *et al*. (2015) showed that the adding of 20 g/kg of red algae (*Pyropia yezoensis*) extract to the diet resulted in a significant increase in the growth rate and weight gain of olive flounder (*Paralichthys olivaceus*) compared to the control group. Different results in different studies have been attributed to the type of fish species, replacement level, diet digestibility, and different experimental environment conditions (El-daim, 2021). Overall, the results of this research showed that adding eight percent of *spirulina* algae extract to the diet of rainbow trout fry can improve the growth rate, immune response and quality of the fish fry.

References

Abdel-Tawwab, M., El-Saadawy, H.A., El-Belbasi, H.I., Abd El-Hameed, S.A.A. and Attia, A.A., 2021. Dietary spirulina (*Arthrospira platenesis*) mitigated the adverse effects of imidacloprid insecticide on the growth performance, haemato-biochemical, antioxidant, and immune responses of Nile tilapia. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 247, https://doi.org/10.1016/j.cbpc.2021.109067.

Amer, Sh., 2016. Effect of *Spirulina platensis* as feed supplement on growth performance, immune response and antioxidant status of mono-sex Nile Tilapia (*Oreochromis niloticus*). *Benha Veterinary Medical Journal*, 30(**1**). DOI:10.21608/bvmj.2016.31332.

Ansari Fard, F., Rajabi Eslami, H., Shamsaei Mahdajan, M. and Soltani, M., 2017. The effect of *spirulina* (*Arthrospira Platensis*) dietary supplement on the immune system and blood biochemical factors of koi fish. (*Cyprinus carpio carpio*). *Iranian Scientific Journal of Fisheries*, Vol.26, Issue 3, pp. 23-32. DOI: 10.22092/ISFJ.2017.113519.

Asadi, M., Azari Takami, Gh. Sajadi M.M., Moezi, M. and Niroomand, M., 2010. Effect of rotifers enriched with betaine and concentrated diet containing betaine on growth, survival and stress resistance of Indian white prawn (*Fenneropenaeus indicus*). *Iranian Scientific Fisheries Journal*, 3(**19**), 1-10. (In Persian).

Biabani Asrami, M., Sodagar, M., Yousefi Siah Kolrodi, S. and Mazandarani, M., 2016. The effect of *spirulina* powder on growth, survival, total carotenoids of pre-breeding and rearing fish in the larval stage of dwarf gourami fish (*Trighogaster lalius*). *Scientific-Research Quarterly of Fisheries Sciences and Techniques*, 6, 1, pp. 21-35.

Biddy, M., Mashkini, S. and Nazari, K., 2018. The effect of *Spirulina Platensis* supplements on growth, body composition, hematological and immunological indices of grass-carp (*Ctenopharyngodon idella*). *Scientific-Research Quarterly of Fisheries Sciences and Techniques*, 8, 4, pp.96-111.

Biranvand, M., Ghaeni, M. and Velayatzadeh, M., 2014. Effect of *Spirulina* sp. supplement on the growth and nutrition of *Zebra Danio* fish. *New Findings in Biological Sciences*, 2, 3, pp. 215-207.

Byeon, Y., Yool Lee, H., Choi, D.W. and Back, K., 2015. Chloroplast-encoded serotonin N-acetyltransferase in the red alga *Pyropia yezoensis*: gene transition to the nucleus from chloroplasts. *Journal of Experimental Botany*, 66(**3**),709-17. DOI: 10.1093/jxb/eru357. Epub 2014 Sep 2. PMID: 25183745; PMCID: PMC4321536.

Derneckbasi, S., Unal, H., Karayucel, I. and Aral, O., 2010. Effect of Dietary supplementation of different rates of *Spirulina* (*Spirulina platensis*) on growth and Feed Conversion in Guppy (*Poecilia reticulate* Peters, 1860). *Journal of Animal and Veterinary Advances*, 9(**9**), 1395 – 1399.

El-daim, A., El Asely, A., Kandiel, M., Abd El-Gawad, E., Elabd, H., Shaheen A. and Abbass A. 2021. Effect of *Spirulina platensis* and *Azolla nilotica* as feed additives on growth performance, antioxidant enzymes and fecundity of *Oreochromis niloticus*. *Iranian Journal of Fisheries Sciences*, 20(**3**) 846-862. DOI: 10.22092/ijfs.2021.124136.

FAO, 2018. Aquaculture Department, the State of World Fisheries and Aquaculture Food and Agriculture Organization of the United Nations, Rome (2018), 2430 P.

Grosshagauer, S., Kraemer, K. and Somoza, V., 2020. The true value of *Spirulina*. *Journal of Agricultural and Food Chemistry*, 68(**14**), 4109-4115. https://doi.org/10.1021/acs.jafc.9b08251

Guroy, D., Tekinay, A.A. and Davies, S.J., 2012. Use of organically certified yeast in the diet of juvenile rainbow trout (*Oncorhynchus mykiss*), Growth performance, nutrient utilization, and fatty acid composition. *Israeli Journal of Aquaculture-Bamidgeh*, 64.

Hoseinifar, S.H., Sharifian, M., Vesaghi, M.J., Khalili M. and Esteban M.A., 2014. The effects of dietary xylooligosaccharide on mucosal parameters, intestinal microbiota and morphology and growth performance of Caspian white fish (*Rutilus frisii kutum*) fry. *Fish and Shellfish Immunology*, 39, 231–236.

Hosseini, M., Kalengi, H., Azadi, H. and Amiri, A., 2017. Comparison of some non-specific immune parameters of skin mucus of yellow bullfish and spotted bullfish. Quarterly scientific research journal

of animal environment. 10th year. Number 3. 270-265.

Hu, Q., 2004. Microalgal cell-mass and Secondary Products –Major Industrial Species *Arthrospira* and *Spirulina platensis*. In: Richmond, A. (Ed.). Handbook of Microalgae Culture: Biotechnology and Applied Phycology. Blackwell Science. pp. 264- 272.

James, R., Sampath, K., Nagarajan, R., Vellaisamy, P. and Manikandan, M.M., 2009. Effect of dietary *Spirulina* on reduction of copper toxicity and improvement of growth, blood parameters and phosphatases in carp, *Cirrhinus mrigala* (Hamilton, 1822). *Indian Journal of Experimental Biology*, 47(**9**), 754-759.

Javed, M., Durrani, F.R., Hafees, A., Khan, R.U. and Ahmad, I.,2009. Effect of aqueous extract of plant mixture on carcass quality of Broiler chicks. *ARPN Journal of Agriculture Biology Science*, 4, 37-40.

Kermani, P., Babaei, S., Abedian-Kenari, A. and Hedayati, M., 2020. Growth performance, plasma parameters and liver antioxidant enzymes activities of Rainbow trout (*Oncorhynchus mykiss*) juvenile fed on *Spirulina platensis* extract. *Iranian Journal of Fisheries Sciences*, 19(**3**), 1463-1478. DOI: 10.22092/ijfs.2019.120209.

Meshkat Roohani, A., Fallahi Kapoorchali, M., Abedian Kenari, A., Sayyad Borani, M. and Zorriezahra, M.J., 2020. Hematite-biochemical and immune response of Caspian brown trout (*Salmo troutta caspius*, Kessler, 1877) juveniles fed different levels of *spirulina* (*Spirulina platensis*). *Iranian Journal of Fisheries Science*, 19(**3**), 1153-1174. DOI: 10.22092/ijfs.2019.119703.

Mohammadiazaram, H., Maniat, M., Ghobanijezeh, K. and Ghotbeddin, 2020. Efffects of *spirulina* powder (*Spirulina platensis*) as a dietary additive on Oscar fish, *Astronotus ocellatus*: assessing growth performance, body composition, digestive enzyme activity, immune-biochemical parameters, blood indices and total pigmentation. *Aquaculture Nutrition*, 27, 252-260. DOI: 10.1111/anu.13182.

Mustafa, Md. G., Umini, T. and Nakagawa, H., 2007. The effect of *Spirulina* feeding on muscle protein deposition in red sea bream, Pagrus major. *Journal of Applied Ichthyoligy*, 10 (**2-3**), 141-145.

Nasrollahzadeh, A., Alaf Navirian, H., Khosh Khalq, M., Musapour Shajani, M. and Shakurian, M., 2013. The effects of Spirulina platensis levels in diet on growth indices and chemical body composition of Kutum fry (*Rutilus rutilus kutum*). Journal of Fisheries (Iranian Journal of natural resources), Vol.67, Issue: 3, pp 445-453.(In Persian).

Nekuiefard, A., Dadger, S., Hafezieh, M. and Nahavandi, R., 2017. Investigating the survival rate of the effect of feeding Caspian salmon larvae (*Salmo trutta caspius*) with

the decapsulated cyst of *Artemia urmiana*. *Animal Environment*, 10(**4**), 251-256. (In Persian)

Niroomand, M., Sajadi, M., Yahyavi, M. and Asadi, M., 2011. Effects of dietary Betaine on growth, survival, body composition and resistance of fry Rainbow Trout (*Oncorhynchus mykiss*) under environmental stress. *Iranian Scientific Fisheries Journal*, 20(**1**), 135-146.

Olvera-Novoa, M.A., Dominguez-Cen, L.J., Olivera-castillo, L. and Martinez-Palacios, C.A., 2008. Effect of the use of the microalga *Spirulina maxima* as fish meal replacement in diets for tilapia, *Oreochromis mossambicus* (Peters), fry. *Aquaculture Research*, 29, 10, 709-715. https://doi.org/10.1046/j.1365-2109.1998.29100709.x

Phromkunthong, W. and Pipattanawattanakul, A., 2005. Effects of *Spirulina* sp. on growth performance and antibody levels in hybrid catfish, *Clarias macrocephalus* x *Clarias gariepinus* (Burchell). *Songklanakarin Journal of Science and Technology Aquatic Science*, 27(**1**), 115-132.

Promya, J. and Chitmanat, C., 2011. The effects of *Spirulina platensis* and Cladophora Algae on the Growth Performance, Meat Quality and Immunity Stimulating Capacity of the African Sharptooth Catfish (*Clarias gariepinus*). *International Journal of Agriculture & Biology*, 13(**1**), 77-82.

Raji, A.A., Jimoh, W.A., Abu Bakar, N.H., Mohd Taufek, N.H., Muin, H., Alias, Z., Milow, P. and Abdul Razak, S., 2020. Dietary use of *Spirulina* (*Arthrospira*) and *Chlorella* instead of fish meal on growth and digestibility of nutrients, amino acids and fatty acids by African catfish. *Journal of Applied Phycology*, 32, 1763–1770.

Sadeghi Gogri, S., Pikan Sireti, F. and Darafshan, S., 2019. The effect of nutritional supplement lactoferrin and *spirulina* algae powder on growth and immunity indicators of female zebra fish (*Zebra Danio*). *Veterinary Research and Biological Products* DOI:10.22092/VJ.2020.343081.1726 . pp. 1-14.

Salighezadeh, R., Yavari, V., Mohammad Mousavi, S. and Zakari, M., 2014. The effect of different levels of *Spirulina Platensis* dietary supplement on some indicators of growth, nutrition and biochemical composition of the body of *Mesopotamichthys sharpeyi*. *Journal of Aquatic Ecology*, 5(**1**), pp. 44-50.

Sarsangi Aliabadi, H., 2018. Investigating the effect of feeding shifts and release size on the growth and food conversion ratio of rainbow trout in Bafaq brackish water. Saltwater Fish Research Station, Iran Fisheries Research Institute. Final Report of the Research Project, number Frost 146.88, 46 P.

Schoonjans, F., 2008. MedCalc Statistical Software (Version

9.5.2.0). [Computer software]. 3d2f.Com. http://3d2f.com/programs/13-638-medcalc-statistical-software-download.shtml.

Sharifi Asl, M. and Rumiani, L., 2021. Optimizating the use of hydroalcoholic extract of *Tetraselmis suecica* algae in reduction of yellowfin tuna oil oxidation using response surface analysis. *Journal of Fisheries (Iranian Journal of Natural Resources*), 76, 1, pp.103-110. DOI: 10.22059/JFISHERIES.2021.342004. 1326. (In Persian).

Sheikhzadeh, N., Mousavi, S., Hamidian, G., Firouzamandi, M., Khani Oushani, A. and Mardani, K., 2019. Role of dietary *Spirulina platensis* in improving mucosal immune responses and disease resistance of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 510, pp. 1-8. https://doi.org/10.1016/j.aquaculture.2019.05.009.

Shugar, D., 2000. The measurement of lysozyme activity and ultraviolet inactivation of lysozyme. *Biochemica Biophysica Acta*, 8, 302–309.

Sirakov, I., Velichkova, K. and Nikolov, G., 2012. The effect of algae meal (*Spirulina*) on the growth performance and carcass parameters of rainbow trout (*Oncorhynchus mykiss*). *Journal of BioScience and Biotechnology*, 151-156.

Sodagar, M., Khalsa, M., Mazandarani, M., Hosseini, S.A. and Zikriai, H., 2015. The effect of *Spirulina* sp on the growth, survival and coloration indicators of *Pseudotropheus demasoni* in fisheries. *Journal of Natural Resources of Iran*, 69, 1, pp. 21-27.

Statistical Yearbook of Iranian Fisheries, 2021. Statistical Yearbook of Iranian Fisheries 1394-1399. Planning and Budgeting Office, (Editor: Gurbanzadeh, R.A. and Nazari, S.), Iran Fisheries Organization, Deputy Planning and Resource Management/Planning and Budgeting Office, Planning and Statistics Department. 64 P.

Taghavi Takyar, M.B., Khajavi's truth, Sh. and Safari, R., 2016. Comparison of antioxidant properties of alcoholic extract of *Spirulina Platensis* and Chlorella Vulgaris algae in laboratory conditions. *Caspian Sea Aquatic Journal*, Second year, 3, pp. 11-18.

Takami, G., Meshkini, S., Rasouli, E., Amini, F., 2005. The nutritional effects of vitamin C-enriched *Artemia urmiana* nauplii on growth, survival rate and resistance to environmental stresses in Rainbow trout (*Oncorhynchs mykiss*) larvae. Pajouhesh and Sazandegi, 66: 25-32. (In Persian).

Takeuchi, T., Lu, J., Yoshizaki, G. and Satoh, S., 2002. Effect on the growth and body composition of juvenile tilapia *Oreochromis niloticus* fed raw *Spirulina*. *Fisheries Science*, 68, 34-40.

Teimouri, M., Keramt, A.A. and Yeganeh, S., 2013. The effects of

Spirulina platensis meal as a feed supplement on growth performance and pigmentation of rainbow trout (*Oncorhynchus mykiss*). *World Journal of Fish and Marine Sciences*, 5(**2**), 194 -202.

Teimouri, M., Yeganeh, S., Rahimi Mianji, G., Najafi, M. and Mahjoub, S., 2019. The effect of *Spirulina platensis* meal on antioxidant gene expression, total antioxidant capacity, and lipid peroxidation of rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry*, 45(**3**),977-986. DOI: 10.1007/s10695-019-0608-3.

Van Vo, Binh, Muhammad, A.B. Siddik, M.A.B., Fotedar, R., Chaklader, Md.R., Hanif, Md.H., Foysal, Md.J. and Huy Quag Nguyen, H.O., 2020. Progressive replacement of fishmeal by raw and enzyme-treated alga, *Spirulina platensis* influences growth, intestinal micromorphology and stress response in juvenile barramundi, *Lates calcarifer*, *Aquaculture*, 529, https://doi.org/10.1016/j.aquaculture.2020.735741.

Velasquez, S.F., Chan, M.A., Abisado, R.G., Traifalgar, R.F.M., Tayamen, M.M., Maliwat, G.C.F. and Ragaza, J.A., 2016. Dietary *Spirulina* (*Arthrospira platensis*) replacement enhances performance of juvenile Nile tilapia (*Oreochromis niloticus*). *Journal of Applied Phycology*, 28, 1023 -1030.

Watanuki, H., Ota, K., Tassakka, A.C.M.A.R., Kato, T. and Sakai, M., 2006. Immunostimulant effects of dietary *Spirulina platensis* on carp, *Cyprinus carpio*. *Aquaculture*, 258(**1**), 157–163.

Yousefi, P., Soltani, M., Taheri, M. and Mazaheri Nejad Fard, R., 2014. Investigating the effect of oral consumption of *Spirulina Platensis* on the amount of total protein and the electrophoresis pattern of carp serum proteins. *Journal of Veterinary Microbiology*, 10, 1, pp. 57-68.