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Effects of Dunaliella microalgae (*Dunaliella salina)* on different level of IgM Immunoglobulin in rainbow trout (*Oncrohynchus mykiss)*

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ABSTRACT

Main purpose of this research was to study the effects of Dunaliella microalgae on immunological and survival changes of the rainbow trout. It was planned to measure the amount of IgM blood immunoglobulin and find out the occurred changes in survival rate of the fish. Rainbow trouts were separated in five groups and were fed with diets containing 0., 5,7 ,9 and 11 grams of pure dried Dunaliella in each kilogram respectively Blood samples were taken from 25 random collected fish, at the end of first and third months of culture and were send to the laboratory to measure immunoglobulin factor. Results indicated that the levels of IgM immunoglobulin in the blood pelasma of those fish fed with Dunaliella aglae had meaningful statistical differences ($P<0.05$) with those not received any Dunaliella alage. With the increased levels of Dunliella in food, and increased weight, levels of IgM immunoglobulin were also were also increased. Their measured amounts were 10± 1 miligram per deciliter of blood pelasma respectively. No mortality was observed in any of the groups, showing good adaptation with feeding conditions. Paying attention to the obtained results, indicate that Dunaliella aglae containing β- carotene, has favourable effects on immunology, survival and growth of rainbow trout.

Key words: Rainbow trout, immunoglobulin, Dunaliella microalgae (*Dunaliella salina)*

Introduction

Dunaliella is a unicellular, naked biflagellate green algae, and without cellular membrane .It'stipic species is *Dunaliellasalina*. Dunaliella first discovered in 1838 in the Atlantic coast of france by dunal (Dunal,1838), after it's identified by teodoresco in 1905, and it was named dunal. He was the first one who find out that the pigment responsible for the red colouration displayed by *Dunaliellasalina* (Teodoresco, 1905). The algae *Dunaliellasalina* has been on of the most studied members of chlorophycea by 27 species.

The genusDunaliella has, the unicellular green algae which is responsible for most of the primary production inhypersalinenviroments worldwide. β -Caroten on of the naturally pigments with the highly. Prized antioxidant properties (Edge *et al*., 1997), and by reason of antioxidant and anticancer properties, Dunaliella was used to production nutritional supplement and pharmaceuticals (Tim *et al*., 2007).

Feeding by supplement of β-caroten from *D. salina*, by reason of enhancement β-caroten due to complement activity increased, and on the other hand, serum lysozyme activity increased, and the result indicated that body immune levels increased (Amar *et al*., 2004). In the other trial Japanse parrotfish (*Oplegnathusfasciatus)* and spotted parrotfish (*Oplegnathuspunctatus)* larvae were fed with β -Carotensupplement rotifers, that results showed survival rates of β -Carotensupplemented groups of both Japanese and spotted parrotfish higher than the control (Tachibana *et al*., 1997).

In a research effects of Dunaliella microalgae on growth, survival of change, skin and flesh colour, and also antioxidant capacity factors in rainbow trout by Wang *et al* 2006, results showed that β-caroten due to pigments of skin and flesh, weight, survival and antioxidant activity (superoxidas and proxidases) increased. Also the effectsof *Dunuliellasalina* dry powderon growth , immune function and disease resistance were determined in black tiger shrimp *penaeusmonodon*, showed higher weight gain and resistance to white spot syndrom virus infection and also demonstrated significantly higher stress resistance. Thecolourintensity of shrimp, was correlated with the level of *D. Salina* in the diet.

In recent years, the use of*Dunaliellasalina* pasteas a sole food source or in combination with othermarin organisms such as Rotifer and Artemia is now a standard, in aquarium and hatcherie and it was recognized, that beside of weight gain, due to enhancement the colouration, improved lipid content of the marine microorganisms (wang *et al*., 2006).

In this research, two basic and appliable aims including information collection and knowledge earning about Dunaliella algae effects on immunology and physiology of rainbow trout fish and marketing acceptance of it were studied .Since Dunaliella has anticancer and antioxidant effects , it has been thought that weather It's use could be resulted in increasing fish potential in immunology and changes in immunological complements and proxidase indicators.

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Methods and Materials

The experiment was accomplished in Emamzadeh Ali trout hatchery, 75 kilometers north east of Tehran, east side of laar River, during August and September 2009. 450 young France race trouts with average weight of 100 grams were selected and separated in five equal groups and were placed in 5 small cement canals (65 × 100 × 37 centimeters). Water income was 1.5 liter per second for each canal. For 14 days,these were fed with common hatchery diet. These were fed for 90 days with 5 special processed diets, containing 0.0, 5.0, 7.0, 9.0 and 11.0 grams of *Dunaliellasalina*, respectively. They were fed twice daily at 10 and 16 hours, 1.8 percent of their body weight (Bahri, 1388). Hatchery diet which was then mixed with Dunaliella algae and starch, was provided from skretting co. from Italy (table 1).

Table 1: Composition of hatchery basic diet.

Compositions	Percent
Crude protein	43
Crude fat	12
Fiber	2.8
Ash	6.8
Phosphate	0.9

Duanliella algae was collected from hose-e-soltanlake near ghom and was cultured under salinity and light stress situations in biotechnology laboratory of the scientific and Industrial research organization.

Increased salinity from 15 to 30 percent was effective in increasing β-caroten in the algae. Cultured Dunaliella was separated by centrifuging with MAB separator, model 204, with 8400 RPM. SeparatedDunaliella was dried and powdered, containing 30 percent salt. Water in the hatchery was supplied from spring, with a rathercostant temperature (14±1°c), oxygen concentration of 9.5 mg/l and pH value of 7.5.

Sampling took place in a 3 months period. At the start of the experiment. 5 samples were taken from each canal by chance and after 3 months of culture, similar sampling was done in all of thecanals. Samples were were anaesthetized with the extract of clove flower (1:5000). After drying, blood was taken frompeduncle area (Kalbasi, 1378).Blood of each fish was transfered to a small laboratory tube and weretransferedin ice box to Hemmat laboratory. They were transfered to a centrifuge with 4600 PRM and after 10 minutes, their plasma was separated and were Kept in -80°c.

Indicators were measured by using special trade Kits and on the basis of immunoturbidiometric (Thomas, 1998) and Eliza method (Divi and Bernard, 1996) with Roche cobasmira apparatus and accuracy of 0.1 Quantity of results wereanalysed using One –Way –Anova statistical method, and to appoint if there are meaningful differences between obtained average results, Duncan test in level of $P<0.05$ was tested. Kelemogrof smirnof test was used to analyse the quality of the results and leven test was used to control rivalness of the results using SPSS soft ware (Zar,1999).

Results:

Produced results from measuring IgM immunoglobulin for beginning and end of 90 days experiment in average and standard deviation are shown in table 2. It shows that average IgM in the witness sample 1 was lower than witness sample 2, 1.87 and 5.8 respectively. Highest amount of IgM was measured 8.98 mg/dec L in treatment 2 and lowest was 7.24 mg/dec L in treatment 3. Laboratory analysis has been done in 5 repetitions and are shown in table 2.

Table 2: Average differences of IgM immunoglobulin in rainbow trout fed with diets containing different amounts of Dunaliella algae from start (witness 1) up to 3 months of culturing (witness 2 and 4 treatments).

Time	Treatments	IgM in mg/dec L	
		Average	S.D.
Start	Witness 1	1.87	0.29
	Witness 2	5.8	2.23
after 3 months	Treatment 1	8.1	2.16
	Treatment 2	8.98	1.18
	Treatment 3	7.24	1.24
	Treatment 4	8.8	1.06

Obtained results indicated that levels of the amount of **IgM** in the plasma of fishes received Dunaliella algae were not similar and had meaningful differences ($P<0.05$)with witness groups.**IgM** was increased in all treatments received Dunaliella algae, with highest increase in treatment 2. Its amount in the blood plasma of the witness 2 group, had meaningful differences with other groups.

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Results obtained from using One - Way –ANOVA test andcomparsionof the results using duncan's test, isshown as mean + SE in figure 1. Alternate letters show meaningful differences, obtained by using Duncan method.

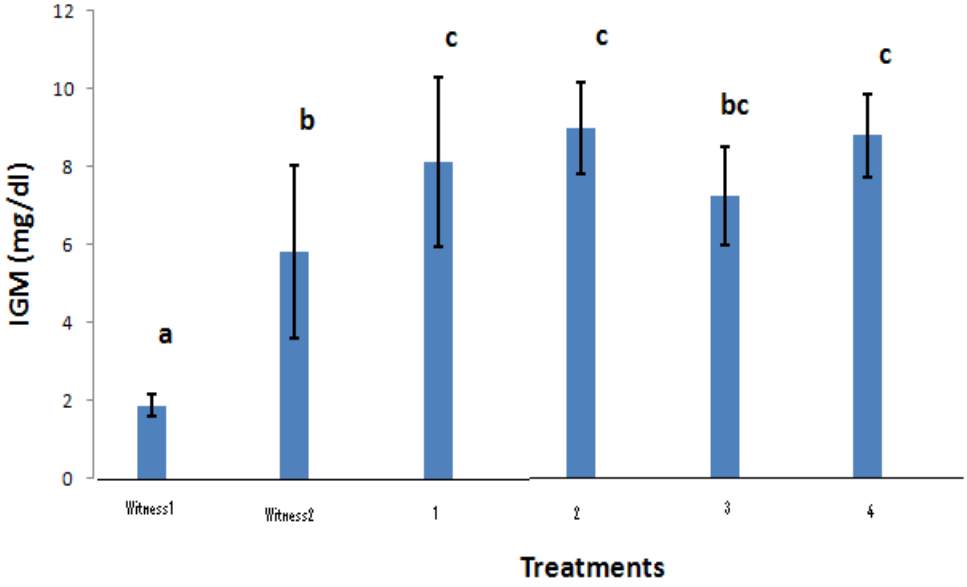

Fig. 1: Average IgM and standard deviations of witnesses and treatments of rainbow trout fed diets containing different amounts of Dunaliella algae.

Biometric results:

Increase in average weight and length of the trouts during 90 days duration of culture are shown in table 3. Increased weight in witness 1 group (102.6 grams) was lower than witness 2 (460 grams). Highest weight increase belonged to treatment 4 (550 grams) and lower one belonged to treatment 1 (450 grams). Average length in witness 1 group with 20.36 centimeter was lower than witness 2. With 2.84 centimeters. Highest length increase belonged to treatment 4 (30.74 cm) and lowest one belonged to treatment 1 (29.2 cm).

Table 3: Average weight and length differences and condition factor for trouts fed with diets containing Dunaliella algae at the beginning and end of experiment.

Measuring Time	Treatments	Averages		Condition factor rate
		Weight in grams	Length in cm	Average
Begining	Witness 1	102.6	20.6	1.22
	Witness 2	460	28.4	1.97
after 3 months	Treatment 1	450	29.2	1.83
	Treatment 2	500	30.2	1.82
	Treatment 3	526	30.9	1.77
	Treatment 4	550	30.74	1.87

Paying attention to Obtained datas from weight increase and Obtained results from Duncan test, it perfectly shows that with increase of Dunaliellaalgein the diet , growth increasedalmost similar in all treatments and witnesses which had no statistical differences ($p<0.05$). Results obtained by using One- Way- ANOVA test and comparing obtained averages by Duncan test, are shown in fig-4. Alternate letters show meaningful differences.

Increased length was also obtained related to the amount of Dunaliella algae in the diet. Highest increase was obtained in treatment 4 and then treatment 3. There was a meaningful difference between all treatments and witness one. No meaningful difference was observed between all treatments ($P<0.05$).

Results obtained by using One- Way- ANOVA test and comparing obtained averages by Duncan test, are shown in fig-5. Alternate letters show meaningful differences.

Obtained results from condition factor indicated that, fish growth was similar in all treatment and no significant difference was observed comparing with witness groups($P<0.05$).

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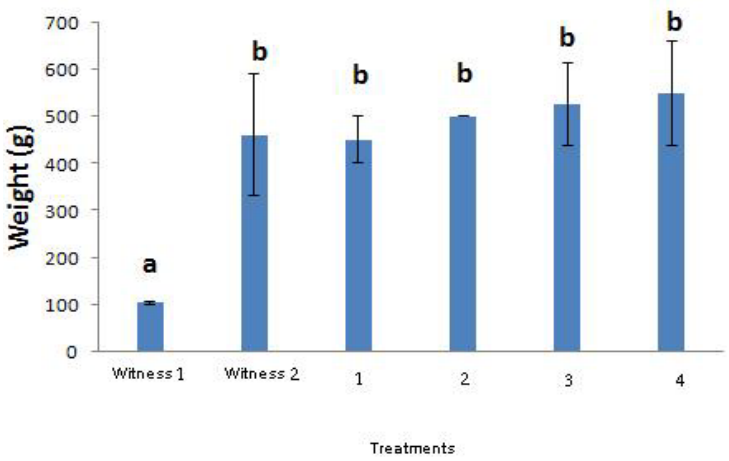

Fig. 3: Average weights and standard deviations of witnesses and treatments of rainbowtrout fed diets containing different amounts of Dunaliellaalgae.

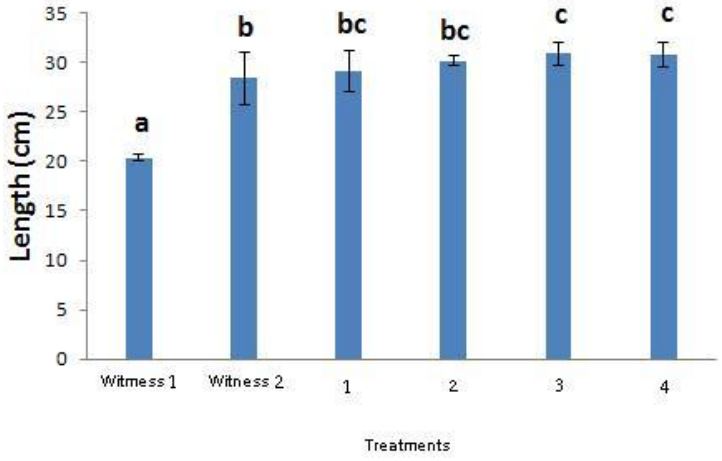

Fig. 4: Average length and standard deviations of witnesses and treatments of rainbowtrout fed diets containing different amounts of Dunaliella algae.

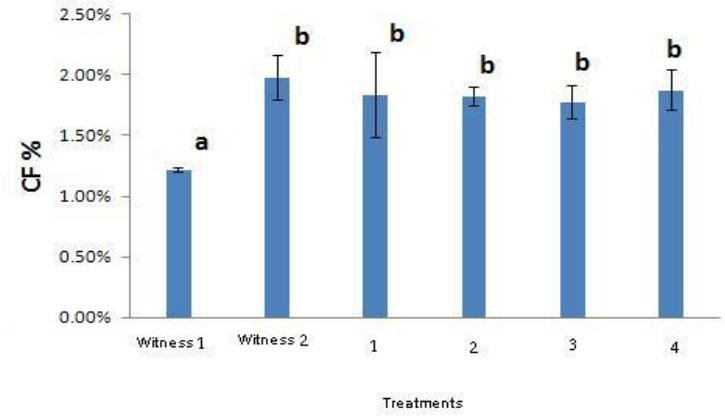

Fig. 5: Average condition factor and standard deviations of witnesses and treatments of rainbowtrout fed diets containing different amounts of Dunaliella algae.

Discussion:

Results indicated that the levels of IgM immunoglobine in the plasma of fishes received dunaliella algae in their diets had meaningful differences (p<0/05) with the witness group. These levels were increased with the increase of dunaliella in the diet and also increase in weight immunoglobine.IgM increase level were 10+1 mg/des liter the plasma respectively. Level of IgM in the plasma of experimental treatments was ascending during the research period ,resulted from increasing of β –Caroten in the diet. Increase of β -Caroten through increase of inductive mitogene, causes increase in aboundance of lymphocytes and T cell (Tachibana *et al*, 1997). Increase in the level of IgM, indicate the connection between FC receptors with IgM , in which receptors are able to identify and digest bacteria covered with IgM antibodies .Lymphocytes are ranked among central defence mechanisms and they have an important effect on appearance of special immunological answers concerning cellular and haemoral ones .Haemoral immunologic answers are done by T lymphocyte mediator . Increasing level of immunoglobin through increase in weight is an indicator of the evolve of blood cells and blood making organs, resulting increase in the level of immunoglobin and increase in the effectiveness in immunological system of the rainbow trout, in which increase lymphocyte T is a cellular immunity answer.

β -Caroten is a prefabricator of vitamin A which through inculocation of mitogene, causes increase in leucocytes, lymphocytes (Daniel, *et al*, 1991) and macrophagues (Tachibana, 1984). Supplementary food containing β -Caroten, increases the number of helping T cells after two weeks, but has no effect on the number of suppressor T cells (Alexander *et al* ., 1985). Searching the effeats of supplementary food containing vitamin A (Thompson *et al* ., 1993). Showed that increasing levels of vitamin A in Atlantic salmon (salmosalar), increased the activity of the complements and lysosymes.

In other words, high levels of vitamin A is effective in increasing the activity of antiprotease serum and also increase Phagocytosis and antibacterial activities (Thompson *et al*., 1985).

In this research, it was shown that β -Caroten in dunaliella algae increases also the amount of IgA,which is important to notice. It is also recorded that rotifer enriched with β -Carotenhad high effects on lymphocyte aboundance and Survival rate of the larvae of Japanese Parrot fishes, (*Oplegnathusfasciatus*) and (*O.functatus*) spotted one.

Survival rate of fish fed with enriched rotifer with β -Carotenin comparsion with the control group and also lymphocytes of spleen was higher in Japanese and spotted parrot fishes (Tachibana *et al*.,1997).

Immunoglobulines have 4 different isotopes in the mammals but in bony fishes there is only one isotope named IgM(Altinok *et al*.,1998),which acts similar as in higher animals against bacteria and viruses.

In this case fishes show responses with bacteria and viral antibodies ,in a different way with formation of something equivalent with IgM(Mokhayer,1381). Cells producing IgM are present in derm mucus secrition,gills, lymph,thimus,kidney,intestine and gull bladder (Adkison *et al*.,1996).Also their presence in the egg of some fishes such as carp and pelaice,indicate the transfer of immunoglobuline from mother to it's children. Results of different researches show that the amount of Ig in fishes changes due to age and size and is higher in matured ones.

Obtained result of this research showed an increase in the amount of IgM in the blood plasma of fishes with diets containing dunaliella algae after 3 months. This was resulted in nutralising the effects of affecting bacteria and viruses and their secreted toxins and also activating the complement systems through secondary routes and facilitate swalloing minute particles in which IgM has an important role in it(Bernstein *et al*.1998). The effects of dunaliella algae on the immunological indicators was experimented by Amar *et al*.,in 2004. The effects of this algae containig β-Carotenchromatophores and the ferment *phaffiarhodazyma* containing astaxanthin on non specific protective mechanisms in rainbow trout indicated that carotenoid chromatophores increase the complement and lysosym activities and results in increasing the total number of foreign consuming cells.The amount of immunoglobuline in the plasma of fishes consuming diets containing dunaliella algae will increase (Amar *et al*., 2004).

Attention to the obtained results indicate that immunological changas in rainbow trout will increase by in creaseddunaliella in their diet.this shows that existing B caroten in dunaliellaadgae stimulate immunological system in rainbow trout and has positive effects on its resistance.

Owing to the obtained results and with the attention of rainbow trout being the main culturing fish in Iran,usingdunaliella algae in its diets,in order to provide natural immunity is important.

Since the algae can be provided easily in Iran, it should be suggested to add suitable amount of it to the diet of all farming rainbow trouts in the country.

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