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Effects of dietary marine microalgae *Chlorella salina* on the growth, haematological, digestive enzyme, antioxidant activities and coloration of the freshwater ornamental fish Flame Red Gourami (*Tricogasterlalius)*

Mayavan Veeramuthu Rajeswari[1]*, Dhatchanamoorthi Pattabiraman[1], Eswaramoorthy Thirumalaikumar[1],Tamilvanan Nivetha[1],Ernest Finlay Emmanuel[1]**, Abinaya Gayathri[2],**

[1]Centre for Aquaculture, Sathyabama Institute of Science and Technology, Rajiv Gandhi Road, Jeppiar Nagar, Chennai - 600119, Tamil Nadu, India.

[2]Department of Orthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India.

Corresponding author: Mayavan VeeramuthuRajeswari (raji.shwehari@gmail.com)

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Abstract

Ornamental fish trade is based on their brilliant colors and patterns, which frequently determine the fish's market worth. The aim of the study was to evaluate the effect of dietary marine microalgae *Chlorella salina* on the growth, digestibility, hematological, antioxidant enzyme activities and coloration of *Tricogasterlalius* (Flame red gourami). The experiment trial was conducted for 60 days by feeding different formulations of *C. salina* (100% algal feed (group I), algal feed mixed basal diet in the ratio of 1:1 (group II)) and a control diet with no microalgae added for comparison. From the results, the dietary inclusion of *C. salina* rich feed showed its effectiveness in enhanced growth performance, digestive enzyme, hematological and antioxidant activities in *T. lalius* when compared to the control. Color measurement lightness L* (70.19±0.12), Redness a* (4.51±0.23) and Yellowness b* (14.7±0.22) values were maximum found in *T. lalius*fed with *C. salina* rich diet after 60 days of feeding trials. It was found that increasing the amount of *C. salina* in the fish diet increases carotenoid levels in *T. lalius* skin. This study suggests that *C. salina* rich diet might be used as a necessary feed supplement as well as a coloring agent in the culture of ornamental fish *T. lailus.*

Keywords: *Tricogasterlalius*, *Chlorella salina*, skin color, CIElab, Ornamental fish.

1. Introduction

The ornamental fishes are among the most popular attractions in the world because of their beautiful colors, distinctive patterns and ability to adapt to a confined environment (Rajeswari et al. 2014). In recent years, the marine ornamental trade has increased and developed into a multibillion-dollar industry. It is estimated that over 2 million aquarium hobbyists across worldwide purchase more than 20 million marine ornamental fish from wild collections (Wabnitz 2003). The global ornamental fish trade is estimated to be worth more than US 15 billion dollars, including US 300 million dollars traded in marine ornamental fish (Satam et al. 2018; Pouil et al. 2020).

The price of ornamental fish on the global market is largely influenced by a variety of criteria, including color. Poor color performance in juveniles and adults may also result in price reduction, although this can easily be regulated by the use of a carotenoids-rich supplemental diet (Shahidi and Brown 1998). They also show their body colors for a variety of reasons. including identification, communication, camouflage, mimicry, defense, and adaptation to their environment (Saxena 1994). The fish's body colors were dependent on the special cells found in tissue known as chromatophores. There are four major categories of pigments such as melanins, carotenoids, pteridines and purines, that can be used to provide colors in these cells (Anderson 2001).

Carotenoids are important pigmenting molecules that are normally not synthesized by fish; instead, they must be obtained via eating algae, coral, or wild that have collected these pigments (Devi et al. 2016). Plants, algae, as well as bacteria and some yeast can biosynthesize the carotenoids(Pizarro and Stange 2009). The red, orange, and yellow color that can be found in fish and crustaceans are caused by carotenoids (Packer 1992). Ornamental fish traders are looking for ways to improve the color of their skin. Additionally, they are searching for natural pigments to replace synthetic pigments because synthetic pigments are more expensive(Ramamoorthy et al. 2010).

Dietary supplements of carotenoids are significantly influencing the fish color, and also essential nutrients for healthy growth and reproduction (Paripatananont et al. 1999; Gouveia et al. 2003).Several studies have investigated the usage of different algae as dietary supplements including *Porphyridium*, *Isochrysis*, *Pavlova*, *Chaetoceros*, *Gracillaria*, *Palmaria* and *Arthrospira* are effective color elicitors in cichlid fish, rainbow trout, fish larvae, bivalve molluscs and various gastropods (O'Connor and Heasman 1997; Kop and Durmaz 2008).

The consumer concern about the safety of using synthetic carotenoids in food has interested food producers to look for potential natural alternatives. As a result, the usage of natural ingredients in food and feed has expanded. Therefore, natural carotenoids generated from microalgae could serve as an alternative to synthetic carotenoids in the aquaculture industry (Pezeshk et al. 2019). The present work was undertaken to evaluate the potential of marine microalgae *C. salina* as a dietary carotenoid source for growth, haematological, digestive enzyme, antioxidant and coloring the skin of ornamental fish *T. lalius* (Flame red gourami).

2. Materials and Methods

2.1 Experimental design

Flame red gourami (*T. lalius*) (Initial weight: 4.26 ± 0.75) was purchased from a commercial aquarium fish farm and was acclimatized to the laboratory conditions. The experiments were conducted for 60 days. The fish were randomly divided into four treatments of 30 fish each and a control group. All the fish were held in transparent glass tanks (50 L, 45 (l) x

30 (w) x 45 (h) cm) with natural water supplied continuously under the experimental condition. Water quality parameters in each tank were checked and maintained at a temperature of 24-26°C, a dissolved oxygen level of 2-4 mg L^{-1}, a pH range of 7.0-7.1, and the unionized ammonia was never found in the concentrations greater than 0.01 mg L^{-1}. The experiment was carried out in a room illuminated by a fluorescent light set to a 12-hour light (1,200 lux) and 12-hour darkness cycle.

2.2 Ethics statement

Even though fish have been used in this study, no animals were stressed or sacrificed in the process, and every effort was made to minimize suffering. Principles of laboratory animal care have been followed, and experiments were carried out by the National Institutes of Health's regulations (Jenkins et al. 2014). This study was approved by the institutional ethical committee, Sathyabama Institute of Science and Technology (Ethical Clearance, Biosafety and Animal Welfare Committee).

2.3 Algal material and culture condition

The marine microalgal strain, *C. salina*was cultured in a fresh convey medium(Anderson 2001) at laboratory conditions and inoculum to fresh convey medium ratio of 1:9 under aseptic conditions. A total working volume of one litre of *C. salina* was cultivated at a temperature of 20-25°C, light intensity of about 2000 Lux, 16:8 h light–dark cycle and supplied with continuous aeration. The algal cultures were collected when they reached the early stationary phase of the first stage of cultivation (15 days) (Chong et al. 2019).

2.4 Experimental Feed Preparation

In this study, three types of diet were formulated. In Group 1, the fish were only fed an algal diet of *C. salina*. In Group II, the fish were fed with a basal diet mixed with algal feed in a ratio of 1:1. In Group III, the fish received only a basal diet. The formulation of the basal diets was prepared using the method described by (Thirumalaikumar et al. 2021), as shown in Table 1. The proximate composition of the control and experimental diets were analyzed as per the standard method (Cunniff and Washington 1997), as displayed in Table 2.

Nutrient (%)	Diet (% dry weight)
	Control
Crude protein	29.52 ± 0.13
Lipid	8.33 ± 0.28
Ash	10.36 ± 0.86
Moisture	5.28 ± 0.55

Table 1. Formulation of the experimental diet (g %).

Ingredients	(g%)
	Basal diet
Fish meal	14
Ragi flour	15
Wheat flour	14
Corn flour	17
Rice bran	10
Groundnut oil cake	18
Tapioca powder	5
Fish oil	5
Vitamin premix[a]	1
Mineral premix[b]	1

Table 2. Proximate analysis

1 g protein = 5.6 kcal; 1g lipid = 9.4 kcal.

a Vitamin (IU or mg per kg premix): Vitamin A, 700000IU; Cobalamin, 200IU; Vitamin D3, 70000IU; Vitamin E, 250 mg; Nicotinamide, 1 g.

b Mineral premix (per kg premix): Cobalt, 150 g; Copper, 1200 mg; Iodine, 325 mg; Iron, 1500 mg; Magnesium, 6000 mg; Manganese, 1500 mg; Potassium100 mg; Sodium, 5.9 mg; Sulphur, 0.72%; Zinc, 9600 mg; Calcium, 25.5%; Phosphorus, 12.75%.

All dry ingredients were powdered, passed through a 650 µm sieve andmixed with water until very soft biscuit-textured dough was obtained. Then the mixture was steam sterilized (autoclaved at 120 °C for 20 min) and pelletized by hand to get pellets of 3 mm size. The pellets were dried in a hot-air oven at 55–60°C for 48 h until the final moisture content was less than 15%. After drying, lutein was mixed with experimental diets with 2.5% fish oil and dried in a hot air oven at 35°C for 30 min. All feeds were stored in opaque containers at 4°C before use.

Fishes were fed at the rate corresponding to 3% of body weight twice a day in all the experiments. Water in the experimental tanks was changed every day to provide the fish with adequate oxygen, remove the faeces matter and uneaten feed. At the end of the experiment, all fishes were starved for one day to take the final wet weight. The observations for color enhancement of the fish were measured initially and at end of the experiment.

2.5 Analysis of biological indices

The biological indices (growth performance, weight gain, specific growth rate, feed utilization and survival rate) of the *T. lalius*were evaluated by the following formulae (Gouveia et al. 2003; Kalinowski et al. 2005).

Body weight gain (BWG) = [{Final body weight (g) - Initial body weight (g)}/Initial body weight].

Specific growth rate (SGR) = [In Final body weight (g) – In Initial body weight(g) / Number of days] x 100.
Feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g).
Survival rate =(Final live fish/Initial live fish) X 100.

2.6 Blood and tissue samples collection

On the 30^{th} and 60^{th}days of the feeding trial, each group of fish were euthanized by bath immersion in50 μlL^{-1}clove oil (Himedia, GRM340) for 5 minutes. After anaesthesia, blood samples were collected from thefish caudal vein using a 1 ml plastic syringecontaining a pinch of anticoagulant (0.5% EDTA) used for haematological assessment.Additionally, organs such as the gut, and muscle were dissected from anaesthetized fishes in each experimental and control group to study digestive and antioxidant analysis (Paulpandian et al. 2023).L^{-1} clove oil (Himedia, GRM340) for 5 minutes. After anaesthesia, blood samples were collected from the fish caudal vein using a 1 ml plastic syringe containing a pinch of anticoagulant (0.5% EDTA) used for haematological assessment. Additionally, organs such as gut, and musclewere dissected from anesthetized fishes in each experimental and control group to study of digestive and antioxidant analysis (Paulpandian et al. 2023).

2.7 Haematological assessment

Haematological parameters such as total red blood cells (RBC), white blood cells (WBC), haemoglobin and haematocrit analyses from the collected blood samples with anticoagulants were analysed by using all combinations of automated haematology analyzer (Aspen/Rapid Diagnostic, India) (Levina et al. 1997).

2.8 Evaluation of digestive enzymes

After the 30^{th} and 60^{th} days of the feeding, digestive enzymes like protease, amylase, and lipase were evaluated in the gut tissues of both the experimental and control groups (Bernfeld 1955; Furne et al. 2005). Total amylase, protease and lipase production were determined using Nutrient agar plates supplemented with 1% (v/v) starch 1% (v/v) skimmed milk and 1% (v/v) of tributyrin, respectively. Culture Petri plates were spot inoculated and incubated at 37°C for 24 hours. After incubation, starch agar plates were flooded with iodine solution, appearance of yellow discoloration revealed amylase production. Skim milk agar plates showed a zone of clearance around the inoculum indicating proteinase production. Likewise, tributyrin nutrient agar plates showed a clear zone around the inoculum revealing lipase production.

2.9 Determination of antioxidant properties

Individual samples of the experimental and control group fish's muscle tissue were homogenized in 10% ice-cold 50mM Tris buffer (pH 7.4). The homogenized samples were centrifuged at 12,000 rpm for 15 minutes at 4°C, with the supernatant used to analyse the enzyme activity such as superoxide dismutase (SOD) and catalase (CAT) activity using a commercially available kit (Sigma-Aldrich,Chemie) according to the instruction of the manufacturer. The absorbance of SOD and CAT activities were measured at 550 nm and 405 nm, respectively. CAT activity (1 unit) is referred to as the amount of the sample required to catalyse the breakdown of 1μmoL of H_2O_2 into H_2O and O_2per minute. Similarly, one unit of SOD activity is referred to as the sample volume required to catalyse the breakdown of one μmoLof O^{2-}into H_2O_2 and O_2per minute.

2.10 Skin Color Measurement

After 30th and 60th days of feedings, the skin color of the *T. lalius* was measured on both sides of the dorsal skin by using a CM5 spectrophotometer (Konica Minolta, Japan) with a 5 mm measuring area based on tristimulus values, L* (lightness), a* (redness) and b* (yellowness). Before taking color measurements, each fish was anaesthetized to reduce stress and placed in a petri dish to minimize handling. According to the CIE standards (C I E 1986), the color parameters were measured, L* value for the lightness factor, which ranges from 0 for black to 100 for white; a* value coordinates the red/green chromaticity; and b* value reflect the yellow and blue chromaticity. Skin color measurements were performed at the initial and the end of the experiment in both the experimental and control group.

2.11 Statistical Analysis

Statistical analysis was carried out with Graph pad Prism 8.0 software package using one-way ANOVA and Duncan's multiple range test analyses at the 5% level of probability. Data is reported as the mean ± standard deviation of triplicates (n=3) with a p-value of 0.05 considered as significant.

3 Results and Discussion

3.1 Growth and Survival

The results showed that the survival rate, specific growth rate and feed conversion ratio were not significantly affected by dietary treatments (Table 3). Fish in all experimental groups gained significant weight and length during the experimental period of 60 days. After 60 days of the feeding trial, the fish were grown normally and showed high activity. The specific growth rate of *T. lailus* fed on the experimental diet was significantly higher than that of the control diet. This finding of survival rate, specific growth rate and feed conversion ratio is consistent with the previous study, dietary supplementation of lutein has increased the specific growth rate in Lake Kurumoi rainbow fish, *Melanotaenia parva* after 56 days of observation. Additionally, *Chlorella* sp. has been used as a good source of lutein (Meilisza et al. 2017). Moreover, dietary supplementation with microalgae such as *Chlorella fusca, Chlorella pyrenoids, Chlorella sorokiniana*improve fish growth and decrease FCR ingrey mullet (*Chelonlabrosus*) (Garcia-Marquez et al. 2022), Common Carp (*Cyprinuscarpio* L.) (Abdulrahman et al. 2019), and juvenile rainbow trout (*Oncorhynchus mykiss*) (Chen et al. 2022) respectively.

Table3. Growth performance, Survival rate and feed utilization of *T. lalius*fed with experimental diets for 30 and 60 days

Days	Diets	CD	G-I	G-II
30 days	Initial weight (g)	4.26±0.44	4.4±0.75	4.32 ± 0.83^{b}
	Final weight (g)	6.4±0.06	7.12 ± 0.42^{a}	8.21 ± 0.09^{b}
	Weight gain	50.23±0.23	61.81 ± 0.53^{a}	90.04 ± 0.87^{b}
	Specific growth rate	7.13±0.34	9.06 ± 0.28^{a}	12.96 ± 0.69^{b}
	FCR	0.07±0.01	0.06 ± 0.02^{a}	0.04 ± 0.01^{b}

60 days	Initial weight (g)	4.26±0.44	4.4±0.75[a]	4.32±0.83[b]
	Final weight (g)	7.4±0.43	8.5±0.65[a]	9.1±0.24[b]
	Weight gain	73.70±0.22	93.18±0.13[a]	110.64±0.31[b]
	Specific growth rate	5.23±0.12	6.83±0.33[a]	7.96±0.13[b]
	FCR	0.10±0.55	0.08±0.40[a]	0.06±0.15[b]
	Survival (%)	85	90	90

Values are represented as mean ± SD (n = 3), Significant difference (p<0.05) vs control

3.2 Haematological parameters

The haematological parameters were significantly higher in the experimental groups (Group I & II) when compared to the control group (Table 4) after 30 and 60 days of feeding. After 60 days of feeding trials, the RBC level was significantly increased in Group II (3.84 X 10^6 mm^{-3}) when compared to other groups. In the same way, Group II had significantly higher levels of WBC (48.80 X $10^3 mm^{-3}$), haematocrit (26.80%), and haemoglobin (8.46 g dl^{-1}) than the other groups. The haematological parameters were significantly increased in the experimental groups when compared to the control group after 30 and 60 days of feeding. Consistent with previous studies, dietary supplementation of β-carotene and phycocyanin extracted from *Spirulina platensis* has increased the haematological parameters in Nile tilapia, *Oreochromisniloticus* when compared with the control diet (Hassaan et al. 2021). In addition, *Trichogastertrichopterus* fingerlings fed with Iron Oxide Nanoparticle conjugated *Camellia sinensis* was shown higher levels of RBC, WBC, haematocrit and haemoglobin when compared to the control (Paulpandian et al. 2023). The observed significant improvements in haematological parameters consistently enhance their immune response and defence mechanism against pathogenic infections. The iron deficiency issue in fish would be resolved by the higher haemoglobin concentrations.

Table 4. Haematological parameters of the *T. lalius*fed with different doses of *C. salina* supplemented (Group1 & II) and control diets after 30 and 60 days

Days	**Haematological parameters**	**Different doses of *C. salina* supplemented diets**		
		CD	**G-I**	**G-II**
30 days	RBC (X $10^6 mm^{-3}$)	2.98 ± 0.22	3.19 ± 0.32[a]	3.28 ± 0.10[b]
	WBC (X $10^3 mm^{-3}$)	35.23 ± 0.28	37.33 ± 0.82[a]	38.20 ± 0.42[b]
	Haematocrit (%)	20.12 ±1.22	22.36 ±0.94[a]	24.80 ±0.26[b]
	Haemoglobin (g dl^{-1})	3.22 ± 0.62	5.37 ± 0.33[a]	5.97 ± 0.46[b]
60 days	RBC (X $10^6 mm^{-3}$)	3.68 ± 0.42	3.79 ± 0.25[a]	3.84 ± 0.20[b]
	WBC (X $10^3 mm^{-3}$)	43.22 ± 0.82	44.20 ± 1.72[a]	48.80 ± 0.48[b]
	Haematocrit (%)	25.82 ±1.29	26.63 ±1.24[a]	26.80 ±0.46[b]
	Haemoglobin (g dl^{-1})	6.52 ± 0.78	7.48 ± 0.28[a]	8.46 ± 0.72[b]

Values are represented as mean ± SD (n = 3), Significant difference (p<0.05) vs control

3.3 Digestive enzyme activity

The digestive enzymes like protease, amylase, and lipase were significantly improved in fish fed a *C. salina*supplemented diet when compared to the control diet (Fig 2a-c). The digestive enzyme activity in fish fed with different concentrations of *C. salina*supplemented diets ranged from 0.8 ± 0.26 (G-I) to 0.85 ± 0.39 (G-II) U/mg Protein for amylase, 1.3 ± 0.21 (G-I) to 1.75 ± 0.19 (G-II) U/mg protein for protease, and 0.33 ± 0.22 (G-II) to 0.42 ± 0.43 (G-I) U/mg Protein for lipase after 60 days of feeding trials. The basal diet mixed *C. salina*(1:1) fed to the fish showed the highest level of amylase and protease activity, whereas the fish fed with *C. salina* showed the maximum level of lipase activity when compared to the control diet (Fig 1a-c). These results agree with the previous study: Nile tilapia (*Oreochromisniloticus*) fingerlings fed with the green microalga *Scenedesmusquadricauda*, which improved activities of digestive enzymes such as lipase, α-amylase, and protease (Abdel-Tawwab et al. 2022a). Likewise, dietary supplementation of *Chlorella vulgaris* has enhanced the protease, amylase, and lipase enzyme levels in *O.niloticus*, when compared to the control (Abdel-Tawwab et al. 2022b). Furthermore, fish growth is influenced by the activity of their digestive enzymes, which is important for nutritional physiology. Additionally, it improves our knowledge of the potential impacts of feed on the growth and consumption of fish (Dawood et al. 2015).

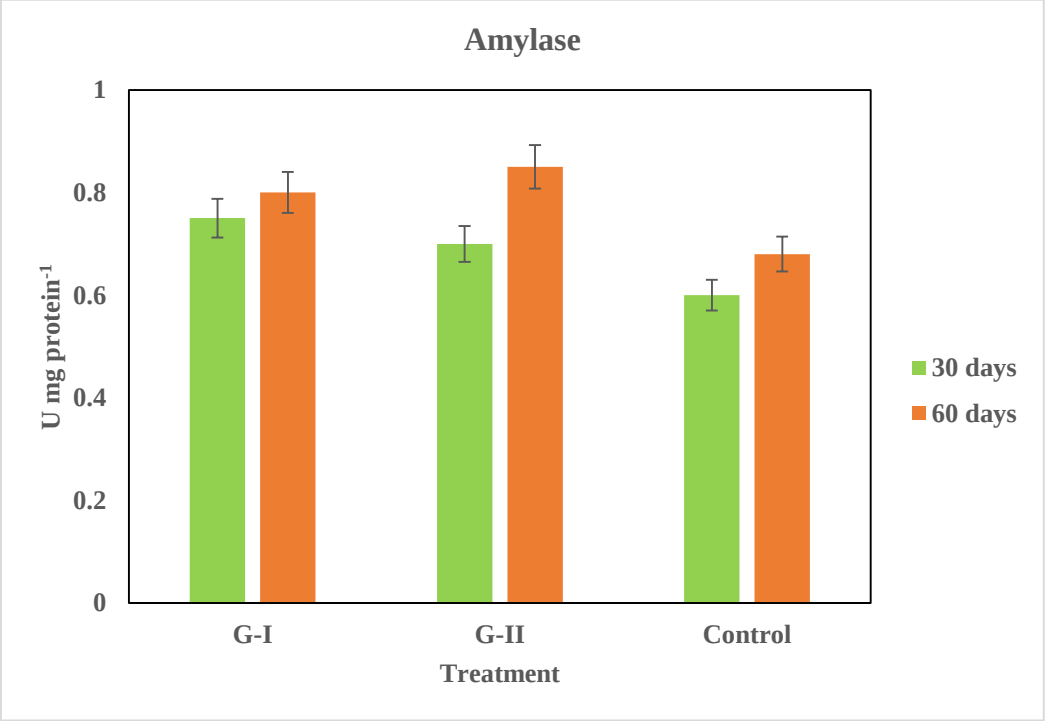

Fig 1a. Amylase enzyme activity in the gut sample of *T. laliusf*ed on control and *C. salina*supplemented diets after 30 and 60 days (means ± SD, n= 3 tanks)

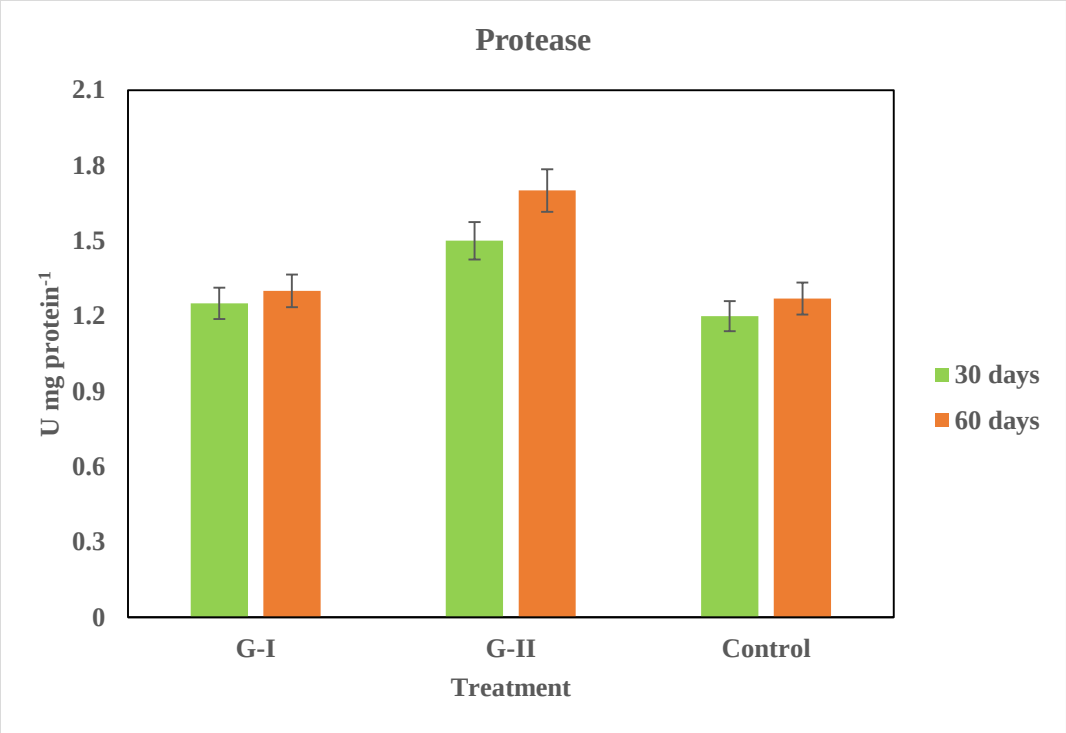

Fig 1b. Protease enzyme activity in the gut sample of *T. lalius*fed on control and *C. salina*supplemented diets after 30 and 60 days (means ± SD, n= 3 tanks)

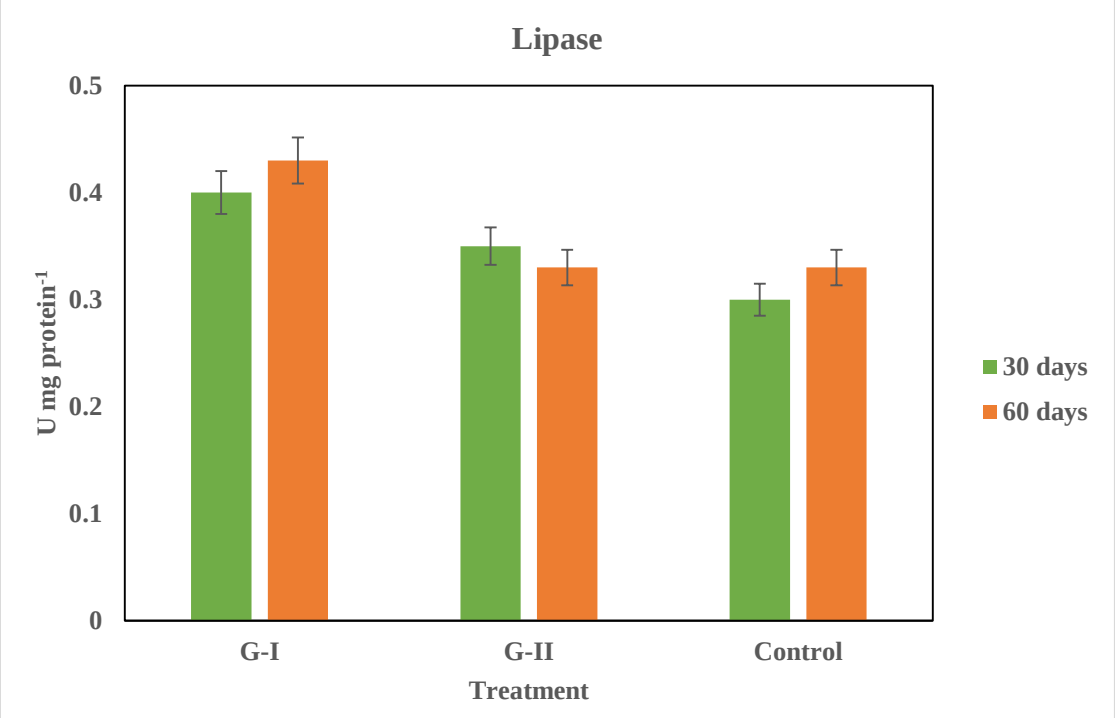

Fig 1c. Lipase enzyme activity in the gut sample of *T. lalius*fed on control and *C. salina*supplemented diets after 30 and 60 days (means ± SD, n= 3 tanks)

3.4 Antioxidant enzyme activity

The result of SOD and CAT activities were shown in Figures 2a and b, respectively. The SOD activity in the *C. salina*supplemented groups (ranged from 20 ± 0.15 (G-I) to 26 ± 0.34 (G-II) U/mg proteins) was significantly lower than the control group (33 ± 0.34 U/mg proteins) after 60 days of feeding trails. Similarly, the CAT activity was observed less in the *C. salina*supplemented groups (ranging from 14 ± 0.15 (G-I) to 15 ± 0.44 (G-II) U/mg proteins) than the control group (27 ±0.45 U/mg proteins). In the treatment group containing basal diet mixed *C. salina* in ratio of 1:1 (G-II), the SOD and CAT activities were slightly higher than the other treatment group ($p > 0.05$). These findings were consistent with the previous study: the SOD and CAT activities of *Labidochromiscaeruleus* fed the diets supplemented with extracts derived from*Sargassumboveanum*, *Gracilariapersica* and *Entromorphaintestinalis*were significantly lower than the control (Pezeshk et al. 2019). According to this study, the usage of algal extract contains antioxidant compounds, which seem to reduce the accumulation of oxidative chemicals in the kidneys and liver and, as a result prevent fish mortality (Aghajani et al. 2015). These findings are consistent with the results obtained from measuring CAT and SOD activity.

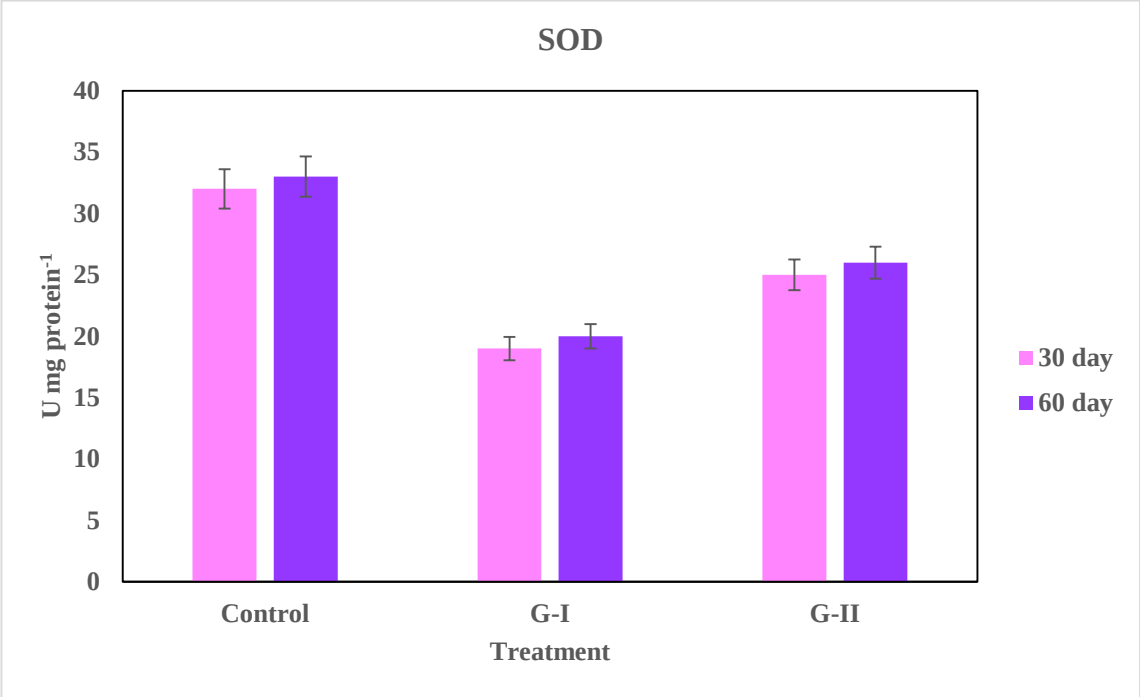

Fig 2a. SOD activity (U mg/protein) of the *T. lalius*fed with different doses of *C. salina* supplemented (Group 1 & II) and control diets after 30 and 60 days (means ± SD, n= 3 tanks).

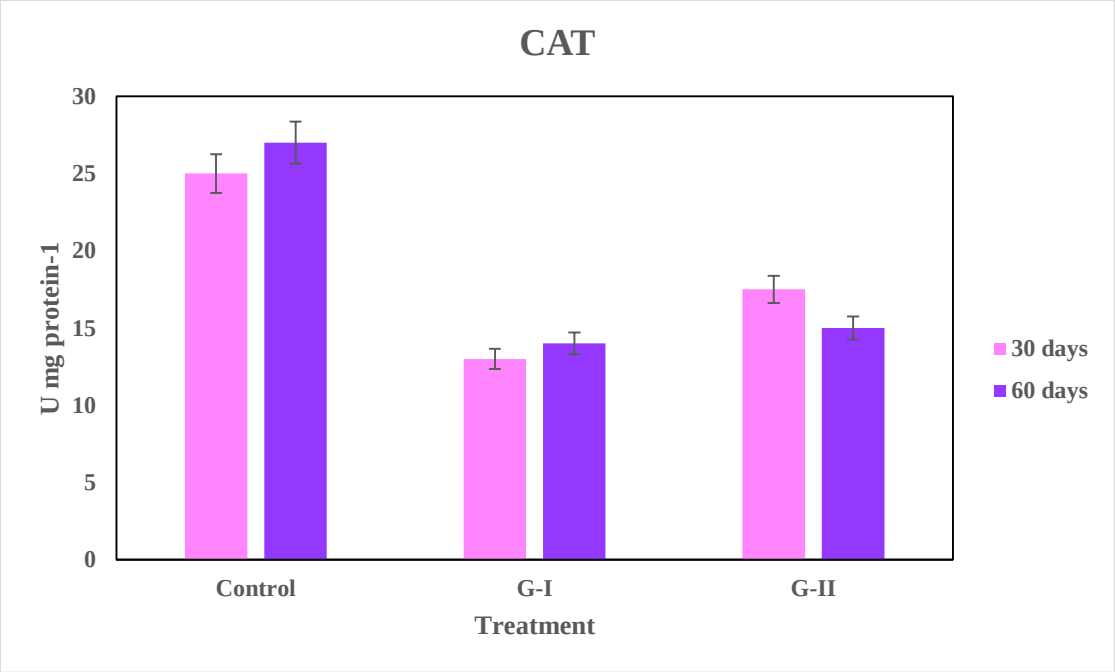

Fig 2b. CAT activity (U mg/protein) of the *T. lalius*fed with different doses of *C. salina* supplemented (Group 1 & II) and control diets after 30 and 60 days (means ± SD, n= 3 tanks).

3.5 Analysis of Skin color

The diets used in this study comprised different concentrations of *C. salina* as coloration pigment. After 15 and 30 days of feeding the fishes those diets, the results revealed that the dietary carotenoids can alter the skin color of the *T. lalius* fish. Lightness (L*), Redness (a*) and Yellowness (b*) values of the dorsal skins of the *T. lalius* were displayed in Fig 3a-c. At the end of the feeding trial, the maximum L*, a* and b* value was found in the treatment group fed with *C. salina* rich diet (G-I) when compared to others, it was 70.19, 4.51 and 14.7 respectively. The diet of microalgae *C. salina* rich feed showed its effectiveness in increased skin red color deposition at end of the 60th day. These results were in agreement with the previous study, in which it was indicated that dietary-supplemented carotenoids can enhance the skin coloration of fish(Pan and Chien 2009; Yasir and Qin 2010). The effect of dietary carotenoids on fish skin color change was more easily visible by direct analysis, indicating that carotenoid-supplemented diets induce skin coloration in *T. lalius.* This was consistent with the results of (Xu et al. 2006) and (Yanar et al. 2008) for goldfish. Moreover, (Pérez-Escalante et al. 2012) and (Liu et al. 2016) have reported that carotenoid supplementation may induce chromatophore production and pigment granules, resulting in increased coloration in fishes. The exposure value (L*) and the yellow-blue color (b *) of the fish were not affected by the experiment diets because the color of the *T. lalius* was mostly dark-orange, which did not affect the values of L* and b*. The characteristic change in redness (a*) value represents the difference in orangeness or brightness of the skin color of fish among the experimental diets. According to earlier research, the skin colour of ornamental fish changes over time based on the amount of carotenoids in the experimental diet (Chatzifotis et al. 2005; Ramamoorthy et al. 2010).

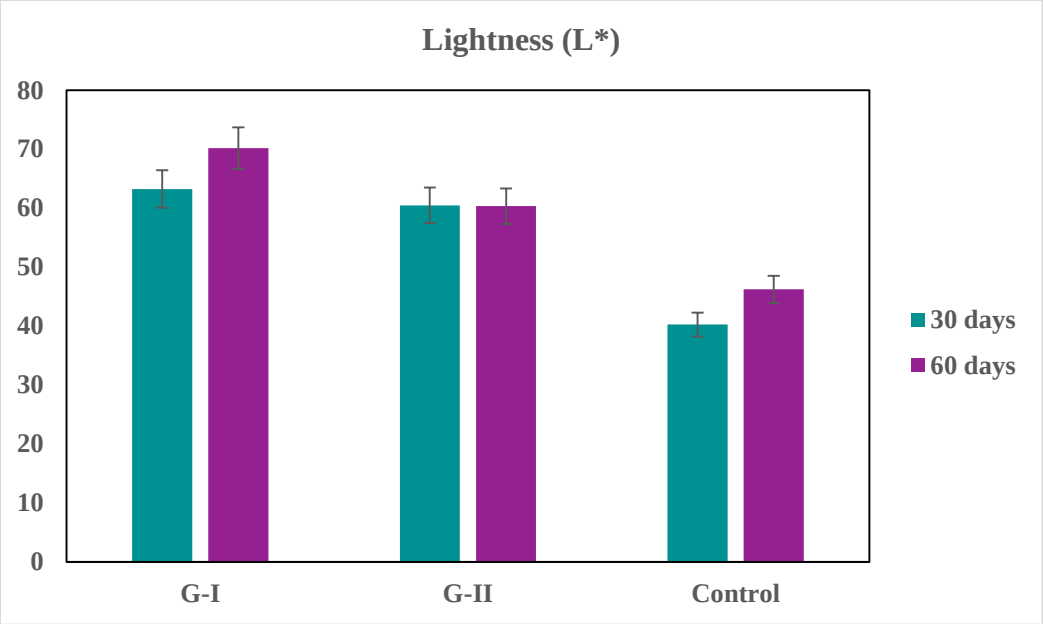

Fig 3a. Lightness (L*) values of the dorsal skin of *T. lalius*fed with different doses of *C. salina* supplemented (Group 1 & II) and control diets after 30 and 60 days (means ± SD, n= 3 tanks).

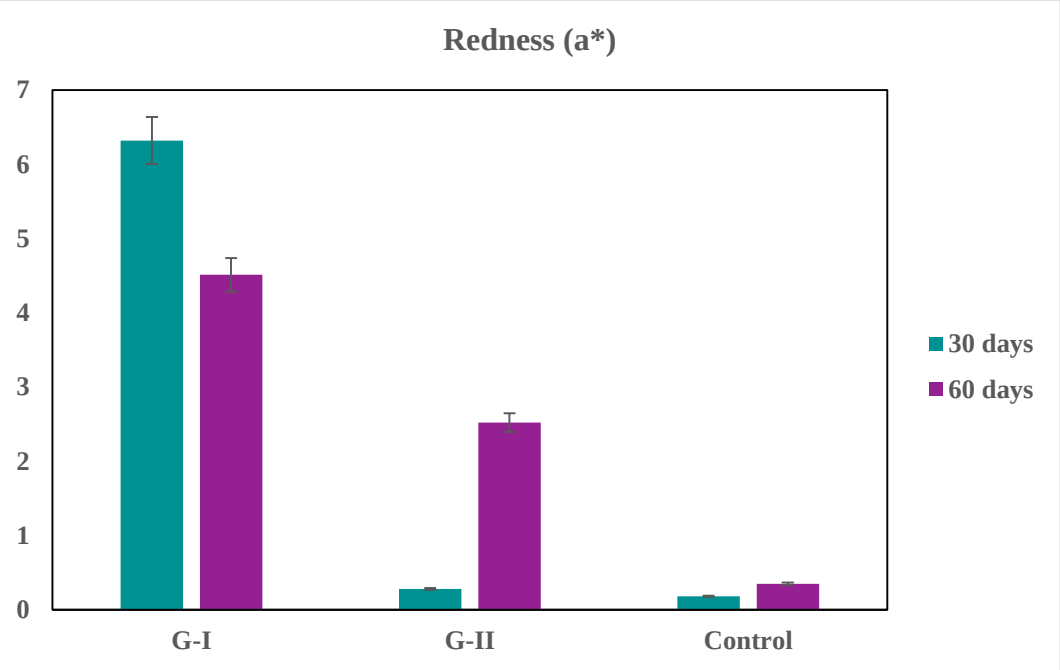

Fig 3b. Redness (a*) values of the dorsal skin of *T. lalius*fed with different doses of *C. salina* supplemented (Group 1 & II) and control diets after 30 and 60 days (means ± SD, n= 3 tanks).

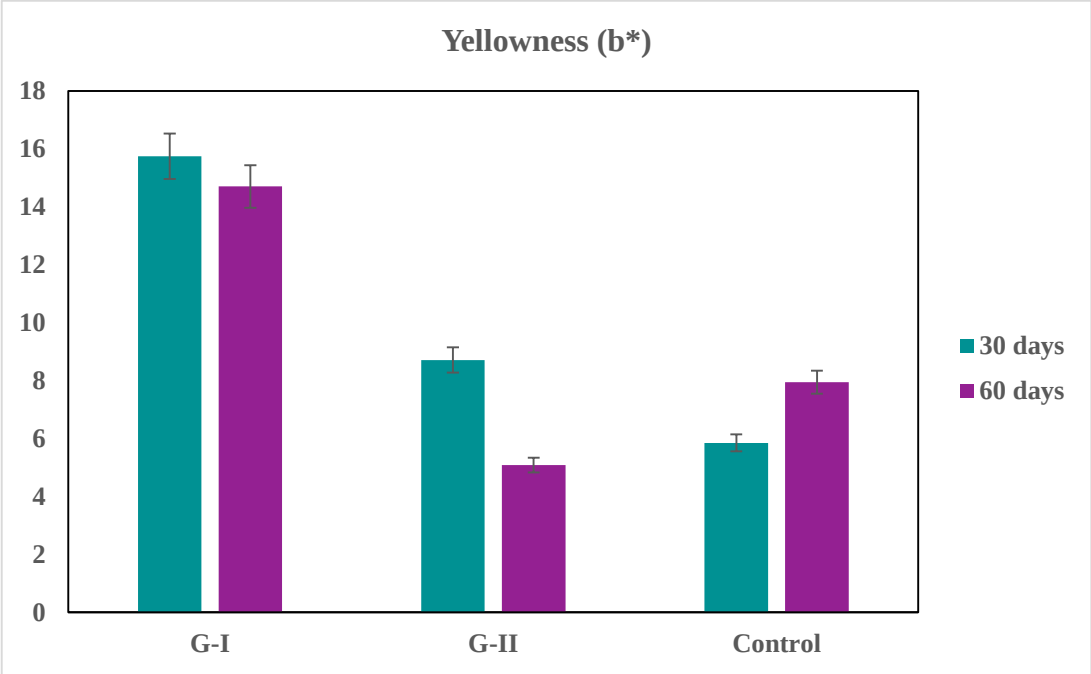

Fig 3c.Yellowness(b*) values of the dorsal skin of *T. lalius* fed with different doses of *C. salina* supplemented (Group 1 & II) and control diets after 30 and 60 days (means ± SD, n= 3 tanks).

4. Conclusions

In conclusion, feeding *T. lailus* with different concentrations of marine microalgae *C. salina* enhanced growth performance, haematological parameters, digestive, antioxidant enzyme activities and pigmentation when compared to control, suggesting that it might be used as a necessary feed supplement in the culture of ornamental fish *T. lailus*.

5. References

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