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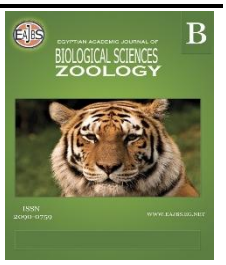

Using *Spirulina platensis* as Feed Additive to Improve Color and Immunity in Goldfish (*Carassius auratus*)

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ABSTRACT

The present study was carried out to assess the effects of *Spirulina platensis* as a feed additive to enhance color and immunity of goldfish (*C. auratus*). Fish (3.5 g) were randomly assigned to eight concrete ponds (8 m^3) with three fish / m^3 capacity, and they were split into four treatments in two replicates. A commercial diet of 30 % crude protein was fed to fish in the four treatments as follow: the control group received no additive of *Spirulina platensis*, T1, T2 and T3 received 6, 8 and 10 g *S. platensis* per kg, respectively. The highest total carotenoids concentration in the skin of *C. auratus* was in T2 (1.32 ± 0.15 µg g^{-1}). While MDA exhibited a considerable decline, antioxidants SOD, CAT, and GPx showed tremendous increases. At the termination of the feeding study, there was no discernible difference between the control and the investigated treatments concerning lysozyme activity, IGM, ALT, AST, and ALP. This discrepancy might be connected to *spirulina's* capability to improve fish health. Histological studies support these results, as no negative changes occurred in the cellular composition of liver tissue.

Therefore, we conclude that using *Spirulina platensis* at a rate of 8 g / kg of feed improves the color quality of goldfish and thus increases their price.

INTRODUCTION

The goldfish *Carassius auratus* is a freshwater fish in family *Cyprinidae* of order *Cypriniformes*. It is commonly kept as a pet in indoor aquaria, and is one of the most popular aquarium fish (Azab *et al.,* 2024).

Native to China, the goldfish is a relatively small member of the carp family (which also includes the *Prussian carp* and the *crucian carp*). It was first selectively bred for color in imperial China more than 2,000 years ago, where several distinct breeds were developed. Goldfish breeds vary greatly in size, body shape, fin configuration and coloration (various combinations of white, yellow, orange, red, brown and black) (Brown *et al*., 2018).

A kind of blue-green algae (*cyanobacteria*), called *spirulina,* is edible to both humans and animals. *Spirulina* species are free-floating, filamentous cyanobacteria characterized by cylindrical, multicellular trichomes in an open left-handed helix. Tropical and subtropical lakes with high pH and high carbonate and bicarbonate concentrations are where they naturally occur (Habib *et al.,* 2008). *Spirulina* occurs in Africa, Asia, and South America. In open-channel raceway ponds, where paddle wheels are used to stir the water, the majority of cultivated *Spirulina* is grown (Habib *et al.,* 2008). *Spirulina* is a common natural addition and food supplement. Spirulina's high protein, fat, vitamin, mineral, chlorophyll, β-carotene, and polysaccharide content makes it a perfect dietary and high-quality drug resource, with carotenoids and C-phycocyanin, two unique natural dyes (Careri *et al*., 2001 and Bingula *et al*., 2016). The β-carotene contained in *Spirulina* has an antioxidant mechanism by Scavenge single oxygen quenching, free radical scavenging and chain breaking (El-Sheekh *et al*., 2023). As more and more people learn about the nutritional, physiological, and pharmacological benefits of natural coloring agents, *spirulina's* usage has increased in tandem with this knowledge. Consequently, *spirulina* a source of natural pigments is finding more and more applications, especially in the food and cosmetics industries (Chethana *et al*., 2015).

Naturally occurring lipid-soluble pigments called carotenoids give many plants and microbes their characteristic red, yellow, and orange hues. They play a key role in photosynthesis and photoprotection as their primary functions. Humans and various other species cannot manufacture carotenoids. Carotenoids are utilized as colorants and flavorings in food and feed, as well as a source of provitamin A in nutritional supplements (Kim, 2015). The health advantages of carotenoids to humans and animals are becoming more apparent. *Spirulina* is utilized as a natural colorant in aquaculture and in fish diet (Challem, 1981). Fish must get their carotenoids from food because, like other animals, they are unable to produce them in their bodies (Kim, 2015). Consequently, carotenoid supplementation is necessary to improve the color of ornamental fish. The profitability of the ornamental fish trade is primarily dependant on the vibrant color of the fish, which is the primary feature of ornamental fish. One of the main determinants of aquarium fish prices on the global market is color (Careri *et al*., 2001). Carotenoids are the primary source of pigmentation on fish skin. In the natural environment, the fish meet their carotenoid requirements by ingesting aquatic plants or through their food chains. Colour-enhancing diets should contain additional natural pigments to enhance the colour of the ornamental fish. Freshwater ornamental fish industry has encountered the issue of fish color fading, particularly when the fish are housed in intense cultivation conditions and kept in captivity for extended periods of time (Pailan *et al*., 2015). The ornamental fish industry is not the only field attempting to improve coloration; several scientists are working to improve the color of food fish's muscles or skins because this is a key determinant of the fish's market price. Even though the fish is the right size, it won't fetch a fair price on the market if its color is subpar. The quality and price of the fish will increase if coloration is enhanced by feeding them pigment-enriched diet. Because of worries about the use of synthetic additives and their expensive cost, recent studies have concentrated on natural molecules as an alternative to synthetic carotenoids. Because of their antistress, antibacterial, growth-promoting, immunostimulating, and antioxidant properties, a variety of natural products, including yeasts, microalgae, and herbal extracts, are recognized to have vital functions in improving the immune system. Furthermore, it has been demonstrated that these natural immunostimulants have less adverse effects. Compared to the commercial ones, they are more affordable (Supamattaya *et al*., 2005 and Salnur *et al*., 2009). *Spirulina* and *Chlorella* are two microalgae species that have drawn special interest because of their immunostimulating properties in vitro and/or in vivo (Miranda *et al*., 1998). Rich in antioxidants such as β-carotene, phycocyanin, tocopherols, and superoxide dismutase (SOD), one of the main functions of *S. platensis* is to scavenge free radicals. It is rich in antioxidants, polyunsaturated fatty acids (PUFA), iron, zinc, magnesium, manganese, selenium, and

vitamins, especially B12, as well as a substantial proportion of protein (up to 70%) (Simsek *et al*., 2007). Because it lacks a cellulose cell wall, *S. platensis* allows fish to benefit from it (Karkos *et al*., 2008). Furthermore, according to (Watanuki *et al*., 2006) it has been demonstrated to improve the carp's immune response (interleukin 1β and TNF-α genes). *Spirulina* and / or cell extracts have recently been shown to improve animal immunity by raising phagocytic activity (Duncan and Klesius, 1996). Furthermore, Harikrishnan *et al*. (2003) found that a hot water extract of spirulina boosted the human immune system by increasing the manufacturing of interferon and cytolytic NK cells. Yeganeh *et al*., 2015 show that rainbow trout diets can be supplemented with 10% *S. platensis* as an immunostimulant.

This experiment aims to improve goldfish color by using *Spirulina platensis* as a feed additive in goldfish *Carassius auratus* fed diets with *Spirulina platensis* 0, 6, 8 and 10 mg / kg in treatments control, T1, T2 and T3.

MATERIALS AND METHODS

This experiment took place at the Central Laboratory for Aquaculture Research (CLAR) in Abbassa, Abu-Hammad, Sharkia Governorate, Agricultural Research Center, Egypt.

Goldfish (*Carassius auratus*) fries were brought from the Central Laboratory for Aquaculture Research hatchery and were acclimated for two weeks prior to the experimentation in concrete ponds (8 m^3). Then after, the acclimation period, fish (3.5 g) were randomly distributed into eight concrete ponds (8m^3) 2 x 4 x 1 m as length, width and depth, respectively, with three fish / m^3 capacity represent 24 fish / pond, representing four treatments, each with two replicates as follows:

Control group: fed on commercial diet of 30 % crude protein without any additives.
T1: given 30 % crude protein feed with 6 g of *Spirulina platensis* / kg
T2: given 30 % crude protein feed with 8 g of *Spirulina platensis* / kg.
T3: given 30 % crude protein feed with 10 g of *Spirulina platensis* / kg.

Cultivation and Harvesting of *Spirulina plantensis*:

In this study, the first isolation of *S. plantensis* was obtained from the botany department of Cairo University's faculty of science.

Spirulina plantensis was cultivated in a phytoplankton laboratory, limnology department of the Central Laboratory for Aquaculture Research. *Spirulina* media (modified) was the media utilized by (Aiba and Ogawa, 1977). White fluorescent light (3000 – 5000 lux) was used to continuously illuminate the culture while it was incubated at 30 ± 2 ºC. During the late exponential development phase, algae were harvested. The pumping system's circulation was halted when the algal culture attained its maximal growth in the stationary phase. The biomass of algal cells was collected using 40 µm nylon fabric, and any salts that were attached were washed away using deionized water. After being dried at 50 ºC in an oven, in an electric coffee grinder, the algal cells (*Cyanophyta*) were pulverized.

Table 1: The experiment's *S. platensis* powder's nutritional value

Nutrients content	Rate %
Protein	61
Fibers	13
Lipids	18
Total carotenoid concentration (µg ml^{-1})	0.204

Over the course of the two-month experiment, fish in each group were fed approximately 3% of their entire body weight twice daily at (8 am and 2 pm). One-third of each pond water

was replaced daily. Water parameters were like as follow: dissolved oxygen level within the range 6.17 – 6.5 mg / l, water temperature 25.8 – 26 °C, both two parameters were measured in site using an oxygen meter (WPA 20 Scientific Instrument), pH 7.3 – 8 which determined in site using a pH meter (Digital Mini-pH Meter, model 55, Fisher Scientific, USA) according to APHA (2000). After the 60 days experimental period, fish were anaesthetized with MS-222, and blood samples were collected from the caudal veins in heparinised tubes. Then, blood samples were centrifuged to obtain the plasma, which was kept in a deep freezer till analyses. The activity of superoxide dismutase (SOD) was assessed using the technique of Flohé and Otting (1984). Activity of glutathione peroxidase (GPX) was assessed using a procedure of Flohé and Gunzler (1984). Catalase (CAT) activity was measured using the method outlined by Beutler (1975). Malondialdehyde (MDA) was determined according to Ohkawa *et al.* (1979). Plasma alanine (ALT) and aspartate aminotransferase (AST) concentrations were measured in accordance with Reitman and Frankel (1957) methodology. Bergmeyer's approach (1974) was used to quantify alkaline phosphatase (ALP). Micrococcus luteus was used as a target in a turbidimetric evaluation of serum lysozyme (LYZ) in accordance with Ellis (1990). One unit of LYZ activity was defined as the quantity of enzyme that caused a 0.001/min reduction in absorption in 1.0 mL of serum. Siwicki and Anderson (1993) calculated total serum immunoglobulin (total Ig) by precipitating Ig with polyethylene glycol and removing the beginning and ending TP. To obtain the liver for the histopathological analyses, the fresh one fish carcass was dissected.

Determining the carotenoids in *Spirulina plantensis*

Carotenoids were extracted using acetone as a solvent. The biomass mixed with acetone in 1: 10 w / v ratio and incubated for three hours at - 10 ºC. Cell debris was removed by centrifugation at 5000 rpm for 10 min at room temperature. The amount of carotenoids was measured using the supernatant. Eq. (3) was utilized to estimate the total carotenoid concentration (Chamovitz *et al*, 1993):

Equation (3) states that carotenoids ($\mu g\ ml^{-1}$) = (A461- (0.046 x A664)) x 4.

At wavelengths of 461 and 664 nm (Unico UV visible spectrophotometer), respectively, the absorbance is denoted as A461 and A664.

Observation of Carotenoid Content in the Tested Fish:

Six fish were taken from each aquarium at random. After being sampled, each of the six sampled fish was placed into a container, frozen at -20 °C, and freeze-dried. The skin of the dried fish was pulled out after forty eight hours, and each sample was mixed with three milliliters of acetone using an HG-300 homogenizer. After being wrapped in parafilm and stored in a dark room for the whole night, at 6000 rpm, the samples were centrifuged for five minutes. An Ultrospec®2000 spectrophotometer (Biochrom Ltd.) was used to assess color intensity at spectro-photometric wavelengths (WL) of 474 nm (Bakshi *et al.,* 2018). Amount of carotenoids in each sample was calculated according to the dry weight of that sample. Total carotenoid content in the skin of the tested fish was estimated according to the following equation:

4 × Optical density value × total sample volume × sample weight (in milligrams).

Histological Analysis:

However, the histology of the liver and gonads was described using the histological techniques of hematoxylin and eosin staining. Following fish dissection, the experimental fish's livers were extracted, and an appropriate piece of each was fixed for the night in a neutral 10 % formaldehyde solution before being exposed to 70 % ethanol. Afterward, they were imbedded in paraffin wax after being dehydrated in ethanol solutions with varying concentrations of ethanol. Sections were cut using a rotary microtome (depot 4 rmt 30 - USA), stained with hematoxylin and eosin, and were 4 – 6 µm thick, and placed on glass slides for examination under a light microscope (Suvarna *et al*., 2012 and Bancroft and Layton, 2019). A microscope (Olympus BX 50) was used to examine and photograph the sections.

Analysis of Statistics:

Before statistical analysis, all data was checked for variance homogeneity and normal distribution using the Kolmogorov-Smirnov and Bartlett's tests. Following that, the GLM process with One-way ANOVA was used to examine the data in accordance with Steel and Torrie's (1980) statistical analysis (SAS, 2009). Significant variations between means were identified using Tukey's test, with a threshold of $P < 0.05$.

RESULTS AND DISCUSSION

Adding *S. platensis* to fish meals had no discernible effect on the water dissolved oxygen levels (6.17 – 6.5 mg / L) or water temperature (25.5 – 26 ◦C), according to water analysis shown in Table (2). (Abdel-Tawwab *et al*., 2021), showed that, this is probably because ponds received artificial aeration and were exposed to the same environmental conditions.

Table 2: Water parameters measured during the experiment.

Parameters	Control	T1	T2	T3	± SE
Temperature ◦C	26^{NS}	25.8	25.9	26	0.25
Dissolved oxygen mg / l	6.17^{NS}	6.45	6.50	6.44	0.2
pH	8^{a}	7.39^{b}	7.3^{b}	7.45^{b}	0.29

Means in the same row having the same superscript letters are not significantly different ($P < 0.05$).

However, three *Spirulina platensis* additive ratios significantly reduced the pH (7.3 – 7.45) levels in pond water in treatments (1, 2, and 3) in comparison to the control group (Table 2). This was in disagreement with the findings of (Mounes *et al*., 2020) who concluded that adding various degrees of *Spirulina platensis* to fish diets didn't affect pH values in fish pond water. Total phosphorus and orthophosphate values were shown in (Table 2). Every water quality measurements found throughout the study fell within the boundaries that are suitable for fish growth, in accordance with (Boyd, 1984).

Carotenoid Concentration in The Skin of *Carassius auratus* at Wavelength 474:

Figure (1), displayed the total carotenoid concentration in *C. auratus* skin at a wavelength of 474 nm. The findings demonstrated that the highest total carotenoid concentration was in T2 (1.32 ± 0.15 µg g^{-1}). The lowest was 0.32 µg g^{-1} at the control feed and T1, respectively.

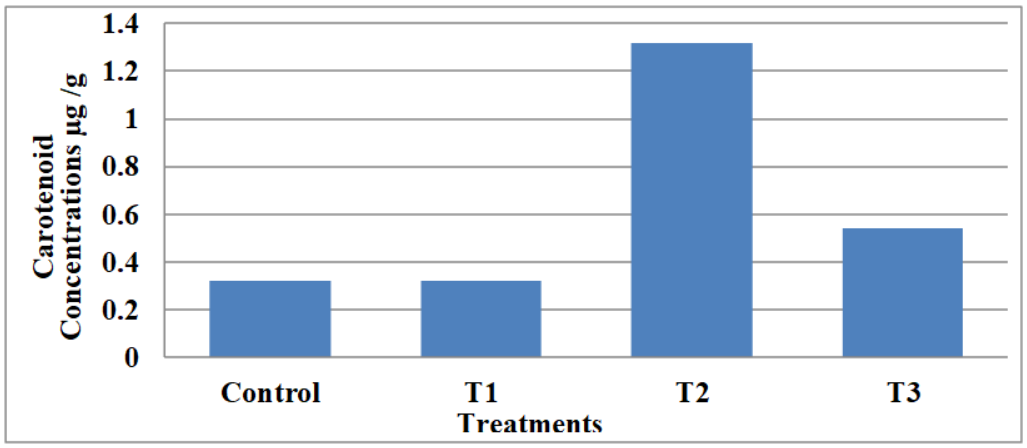

Fig. 1: Carotenoid concentration in the skin of *Carassius auratus* treated with different concentrations of *Spirulina* powder, treated feed, and control feed at wavelength 474 nm.

Only the exogenous feed resources can provide fish with the necessary carotenoids. These feeds mostly consist of different crustaceans, zooplankton, and phytoplankton. These pigments must be provided by the diet because fish have them in their bodies (Johnson and Astaxanthin, 1991). *Spirulina sp*. is a powerful source of carotenoids and significantly influences fish pigmentation where it contains high levels of xanthophylls, β-carotene, and zeaxanthin (Yanar and Tekelioğlu, 1999). The results of the present investigation showed that a meal containing 8 g / kg of *spirulina* powder increased the content of skin carotenoid. (Bakshi *et al.,* 2017) found that feeding *Trichogaster lalius* with spirulina powder has a very good chance of improving its pigmentation. The results showed that feeding the fish 2 g / kg of *spirulina* powder increased the concentration of skin carotenoid at 450 nm and 500 nm wave lengths, respectively, by a significant ($P < 0.05$) margin. But at 6 g / kg, spirulina powder incorporated diet responded with maximum carotenoid accumulation for the wavelengths of 475 nm in the skin of *T. lalius* (Kargın and Dikbaş, 2020) indicted that goldfish that received a 75 mg per kg supplement of *S. platensis* had the best carotenoid ratio.

Growth Parameters:

Table (3), displays the average body weight of the fish for each experimental treatment. T2 (31.68 ± 0.08 g / fish) has the highest final body weight and average daily gain (0.47 ± 0.02 g / fish), while the lowest value (25.08 ± 0.08 and 0.36 ± 0.02 g / fish) was found in the treatment which received no *S. platensis*. Mounes *et al*. (2020) showed that the addition of 10 % *S. platensis* to fish diets resulted in the greatest increase in end weight and average gain / day. Goldfish growth was not significantly impacted by addition of *S. platensis* (25, 50 and 75 mg per kg) to *Carassius auratus* diet (Kargın and Dikbaş, 2020).

Table 3: Growth performance parameters of gold fish in different treatments.

Parameters	Control	T1	T2	T3	± SE
IBW (g / fish)	3.51^{NS}	3.5	3.49	3.51	0.2
FBW (g / fish)	25.08^{c}	28.92^{b}	31.68^{a}	28.8^{b}	0.08
AGD (g / fish)	0.36^{b}	0.42^{a}	0.47^{a}	0.42^{a}	0.02

Means in the same row having the same superscript letters are not significantly different ($P < 0.05$).

Biochemical and Immunological Parameters:

After the experimental period, Plasma alanine (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities, immunoglobulins (IGM) and lysozymes (Lyz) were evaluated in blood serum (Table 4). ALT value was highly significant in the control group (54.5 ± 1.01 IU / L), while the lowest ALT value (23.72 ± 1.28 IU / L) was noted in T3 The control group displayed the greatest values (754.13 and 36.65 IU / L) of AST and ALP, respectively. The lowest value of AST (460.65 IU / L) was recorded in the (T3), while the lowest value of ALP (32.02 IU / L) was recorded in the (T2). The IGM concentration's highest value (468.4 ng / ml) was obtained in T3, while its lowest concentration (271.55 ng / ml) was recorded in T1. Lysozyme's highest concentration (2.45 ng / ml) was achieved in T3, while its lowest value (1.94 ng / ml) was recorded in the control group.

Table 4: Serum levels of immunoglobulins, lysozymes and liver enzymes

	ALT (IU / L)	AST (IU / L)	ALP (IU / L)	IGM (ng / ml)	LYZ (ng / ml)
Control	54.5^{a} ± 1.01	754.13^{NS} ± 50.66	36.65^{NS} ± 3.76	281.1^{NS} ± 51.4	1.94^{NS} ± 0.5
T1	39.29^{ab} ± 5.44	642.3 ± 13.59	35.56 ± 7.9	271.55 ± 43.81	1.97 ± 0.7
T2	24.21^{b} ± 1.067	493.17 ± 98.01	32.02 ± 6.6	415.82 ± 39.41	2.18 ± 0.56
T3	23.72^{b} ±1.28	460.65 ± 85.2	33.9 ± 3.78	468.4 ± 28.92	2.45 ± 0.73

Means with the same superscript letters in the same column did not differ significantly ($P < 0.05$).

Spirulina species are the most widely utilized microalgae for animal feed and food supplements worldwide because of their high nutrient content and affordability at the farm level (Meng-Umphan, 2009). Fish that consume spirulina in their diets develop a tolerance to environmental stress, which may be linked to beneficial chemicals found in spirulina powder (Khorshid *et al.,* 2014).

At the conclusion of the feeding trial, there was an obvious improvement in lysozyme activity in comparison to the control. This rise may be connected to *Spirulina's* capacity to strengthen the immune system by enhancing phagocytic activity (Mahmoud *et al*., 2017). Likewise, earlier research shown that S. platensis might raise Nile tilapia's lysozyme activity (Amer, 2016; Mahmoud *et al*., 2017 and Yilmaz, 2019), Carp (*C. carpio*) (Khalil *et al*., 2017) and Coral trout (*P. leopardus*) (Yu *et al*., 2018). Similar results obtained by Ragap *et al*. (2012), *S. platensis* was administrated orally for four weeks with a dose of 10 mg per fish to *O. niloticus* leads to enhanced lysozyme activities. Likewise, Promya and Chitmanat (2011) found that feeding 3% or 5% dietary spirulina to African sharptooth catfish *Clarias gariepinus* increased serum lysozyme activity significantly. The IgM level activity in the blood of tilapia fed Spirulina was higher than that of fish fed the control diet. This is compatible with Amer (2016), who noticed that 0.5 % Spirulina in tilapia diets increased the IgM. Also, the IgM of tilapia fed 1 g / kg spirulina was increased compared to the control group (Al-Deriny *et al*., 2020). The increased IgM of enhanced immunity in the tilapia body, probably through increasing the local intestinal immunity (Kiron, 2012). Promya and Chitmanat (2011) reported that spirulina enhanced the responses of fish lysozyme activity. Those activities of spirulina could be attributed to its hepatoprotective effects (Mahmoud *et al.* 2018) and content of carotenoids that possess great antioxidant activity (Jensen *et al*., 1998; Hu *et al*., 2008), and of phytopigments as phycobilins, xanthophylls, and phycocyanin antioxidant activity (Bermejo *et al.*, 2008). There was a significant difference in serum ALT and AST levels among the treatments, indicating that the dietary treatments affected the liver functions. The same findings were reported by Velasquez *et al*. (2016) and Al-Deriny *et al*. (2020). Also, Hegazi *et al*. (2014) observed that the adding 10 – 15 % *S. platensis* in tilapia diets significantly decreased the serum levels of these enzymes. However, Abo El-Ward *et al*. (2016) found that 5 – 20 % spirulina increased the serum levels of AST and ALT in tilapia. These results aligned with Karadeniz *et al*. (2009), who claimed that consuming supplements of dietary *S. platensis* enhances liver function by reducing ALT and ALP activity. In our study, T3 showed greater antioxidant enzyme activities, it may have been caused by the phenolic components which having antioxidant properties in the *spirulina* extract, such trans-cinnamic, salicylic, chlorogenic, synephrine, quinic, and caffeic acids (Vasudha *et al*., 2009).

Antioxidant Activity and Immune Status:

In Figure 2, SOD activity showed the lowest value in the control group (48.99 ± 2.89 u / ml), while maximum activity (156.6 ± 2.89 u / ml) was recorded in T2. The number of CAT activity was the lowest (0.777 ± 0.29 u / l) in the control group, but its maximum value (2.96 ± 0.29 u / l) was recorded in T3, as shown in Figure 3. Gpx, as illustrated in Figure 4 also showed the same trend with its lowest and highest activities (55.18 ± 2.89 and 132.2 ± 2.89 u / l), in control and T3, respectively. On the contrary, MDA Figure 5, showed minimal and maximal activities (1.04 ± 0.29 and 3.04 ± 0.29 nmol / ml) in T3 and control group, respectively.

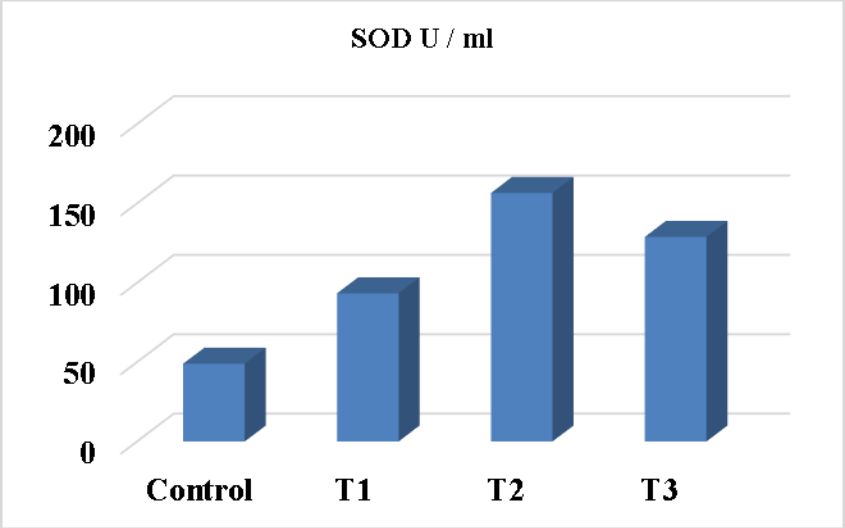

Fig. 2: SOD in goldfish (*C. auratus*) serum; Control group, T1 (6 g *S. platensis*) / kg feed, (T2) (8 g *S. platensis*) / kg feed, and (T3) (10 g *S. platensis*) / kg feed

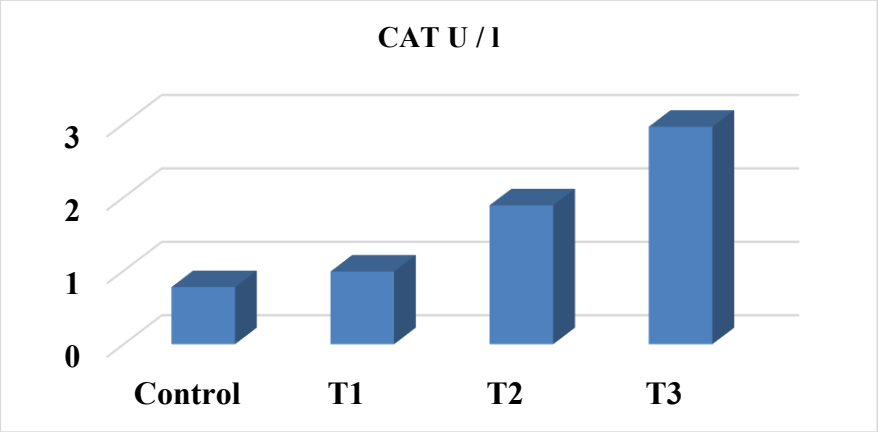

Fig. 3: CAT in goldfish (*C. auratus*) serum; Control group, T1 (6 g *S. platensis*) / kg feed, (T2) (8 g *S. platensis*) / kg feed, and (T3) (10 g *S. platensis*) / kg feed

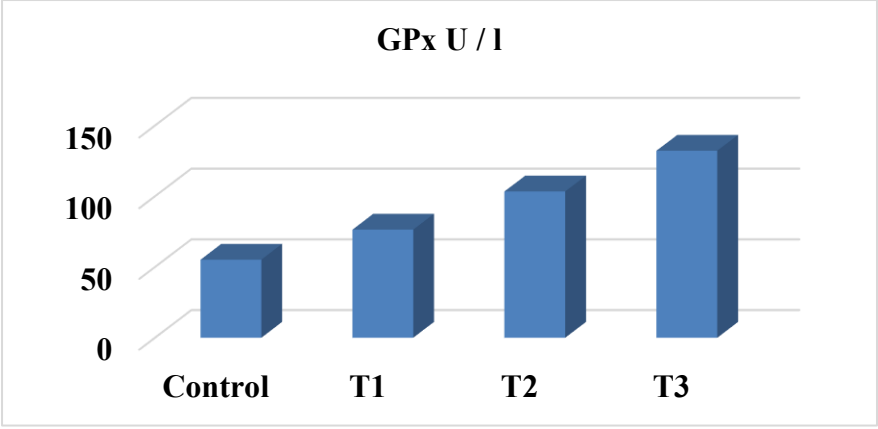

Fig. 4: GPx in goldfish (*C. auratus*) serum; Control group, T1 (6 g *S. platensis*) / kg feed, (T2) (8 g *S. platensis*) / kg feed, and (T3) (10 g *S. platensis*) / kg feed

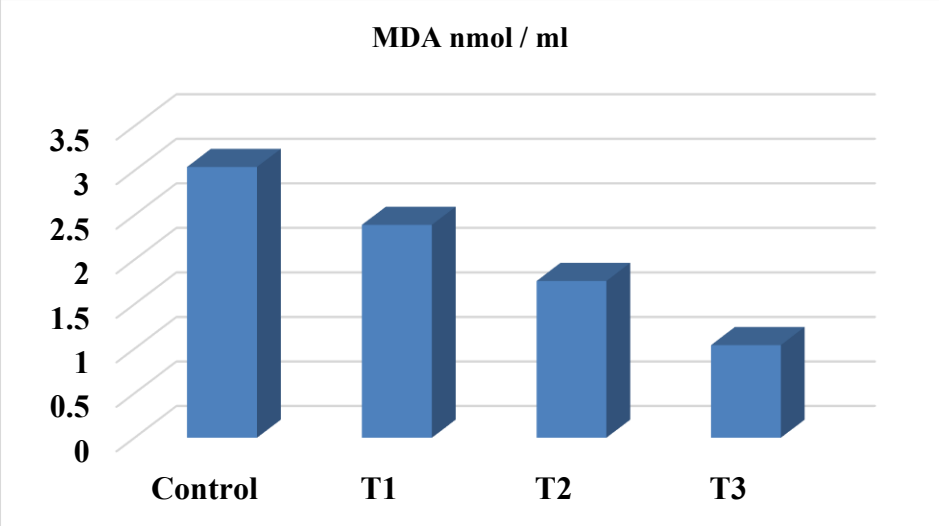

Fig. 5: MDA in goldfish (*C. auratus*) serum; Control group, T1 (6 g *S. platensis*) / kg feed, (T2) (8 g *S. platensis*) / kg feed, and (T3) (10 g *S. platensis*) / kg feed**.**

The antioxidant activity of SOD, CAT, and GPx was considerably higher in fish fed diets supplemented with *S. platensis* than in the control group. Furthermore, MDA levels dropped, which runs counter to the findings of an earlier investigation by Radhakrishnan *et al*. (2014). In the ongoing study, dietary *S. platensis* dramatically increased the activities of antioxidant enzymes such as SOD, CAT, and GPX while decreasing MDA levels. According to Hassaan *et al*. (2021), results are consistent with prior research that attributed these good benefits to the intrinsic antioxidant system's innate ability to scavenge and purify ROS in order to reduce oxidative stress. Due to Liu *et al*. (2010), MDA is the outcome of lipid peroxidation in tissues, which harms both tissues and cells.

The liver in fish has an essential role in detoxification in the body. Any unacceptable changes in the environmental situation and nutrition may have resulted in liver injury (Ahmadmoradi *et al*., 2012). According to Velmurugan *et al*. (2007), these liver abnormalities could be useful indicators of prior exposure to environmental pressure.

Histological Observations:

Liver's sections of goldfish (*C. auratus*) fed diets supplemented with varying amounts of SP showed no histological alterations within the present research. Hepatic sinusoid (HS) Blood vessels (BV) contains red blood cells and the control fish's liver histology (Fig. 6) revealed a normal hepatic parenchyma with regular polyhedric hepatocytes (Hc). Similarly, the hepatic anatomy of goldfish (*C. auratus*) fed a different meal supplemented with SP levels resembled that of controls, displaying normal nuclei and regular polyhedric hepatocytes (Hc) (Fig. 6).

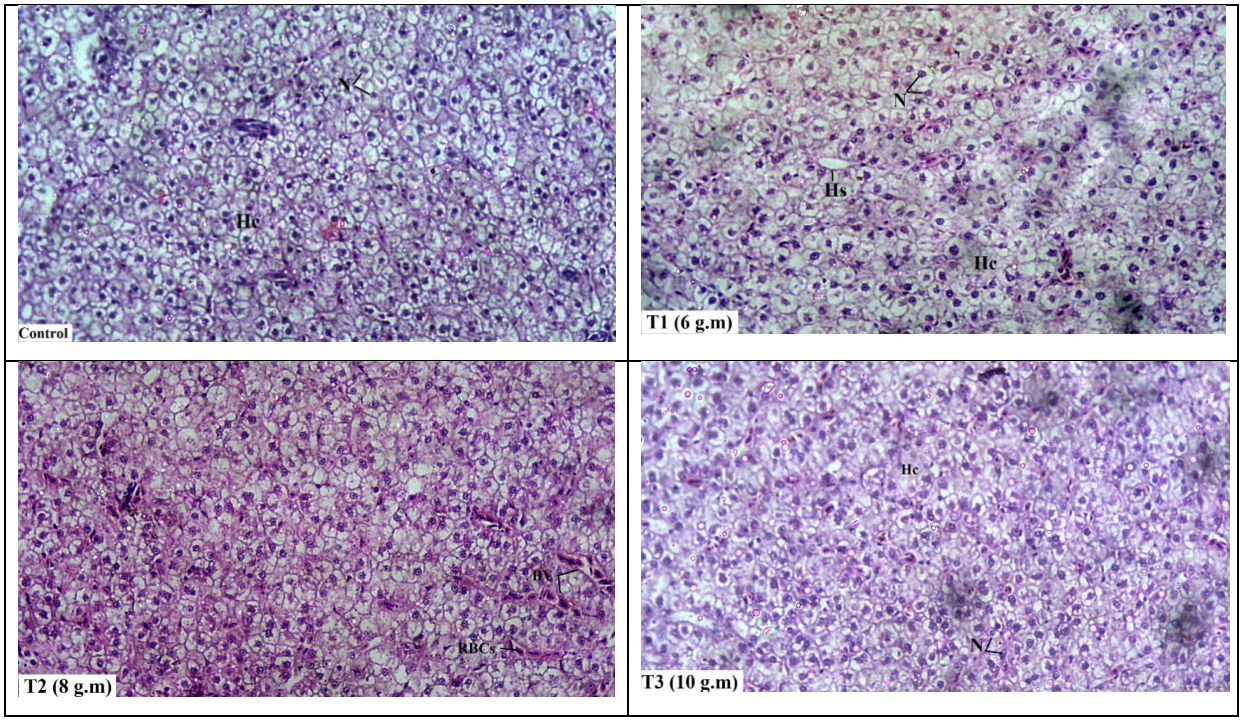

Fig. 6: Histological section in liver of goldfish (*C. auratus*); Control group, T1 (6 g *S. platensis*) / kg feed, (T2) (8 g *S. platensis*) / kg feed and (T3) (10 g *S. platensis*) / kg feed; hepatic sinusoid (HS), hepatocyte (Hc), Blood vessels (BV), red blood cells (RBCs) and Nuclei (N).

According to histological investigation, the current findings also do not reveal any anomalous or histological alterations in the liver, which is consistent with (Abdelrhman *et al*., 2022) who found that, red tilapia fed polysaccharides sourced from Sargassum dentifolium, a brown macroalgae, did not exhibit any liver abnormalities. The bio-sorbents Ulva sp. and *S. platensis* can eliminate toxins off waterways and reducing the detrimental impact of pollution on fish and marine invertebrates its illustrate that the use of algae as feed additives has no adverse effect on fish tissues., according to, Mounes *et al*. (2020). This means that *Spirulina platensis* had useful effect on fish.

Conclusion

Ornamental fish production projects are highly economically viable, but they mostly rely upon the color, physiological state and health of the fish. Employing *Spirulina platensis* as a feed additive in ornamental fish feeds has significantly improved color quality. Therefore, it is recommended to use *Spirulina* at a ratio of 8 g / kg feed to improve color quality of goldfish and increase their price.

Declarations:

Ethical Approval: Not applicable.

Competing interests: The authors declare no conflicts of interest.

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ARABIC SUMMARY

استخدام طحلب الاسبيرولينا بلاتينسيس كإضافة غذائية لتحسين اللون و المناعة في الأسماك الذهبية (*Carassius auratus***)**

ريهام عبد الوهاب عبدالحي[1] , حسام أحمد محمد مؤنس[1], هيثم أحمد جمال[2], رانيا نصر[3], محمد الأحمدي الذهبي[2]

1- قسم اللمنولوجي – المعمل المركزي لبحوث الثروة السمكية – مركز البحوث الزراعية – مصر.

2- قسم التفريخ و فسيولوجيا الأسماك – المعمل المركزي لبحوث الثروة السمكية – مركز البحوث الزراعية – مصر.

3- قسم صحة و رعاية الأسماك – المعمل المركزي لبحوث الثروة السمكية – مركز البحوث الزراعية – مصر.

اجريت الدراسة الحالية لتقييم تأثير طحلب الاسبيرولينا بلاتينسيس كإضافة غذائية لتعزيز اللون و المناعة في الأسماك الذهبية (*C. auratus*). تم توزيع الأسماك (3.5 جم) بشكل عشوائي على ثمانية أحواض خرسانية (8 م^3) بكثافة ثلاث أسماك / م^3، و تم تقسيمها إلى أربع معاملات في مكررين. تم تغذية الأسماك على عليقة صناعية تحتوي على 30 % بروتين خام في المعاملات الأربعة على النحو التالي: لم تتلق المجموعة الضابطة أي إضافة من طحلب الاسبيرولينا بلاتينسيس، المعاملة الاولي: أعطيت عليقة تحتوي على 30 % بروتين خام مع 6 جم من طحلب الاسبيرولينا بلاتينسيس/ كجم، المعاملة الثانية: أعطيت عليقة تحتوي على 30 % بروتين خام مع 8 جم من طحلب الاسبيرولينا بلاتينسيس/ كجم و المعاملة الثالثة: أعطيت عليقة تحتوي على 30 % بروتين خام مع 10 جم من طحلب الاسبيرولينا بلاتينسيس/ كجم. كان أعلى تركيز إجمالي للكاروتينات في جلد السمكة الذهبية (*C. auratus*) في المعاملة الثانية (1.32 ± 0.15 ميكروجرام جم$^{-1}$). بينما أظهر MDA انخفاضًا ملحوظًا، أظهرت مضادات الأكسدة SOD، CAT و GPx زيادات معنوية. عند نهاية التجربة، لم يُلاحظ أي فرق ملحوظ بين المجموعة الضابطة و المعاملات المدروسة فيما يتعلق بنشاط الليزوزيم، IGM ، ALT، AST و ALP. قد يكون هذا التباين مرتبطًا بقدرة طحلب الاسبيرولينا على تحسين صحة الأسماك. دعمت الدراسات النسيجية هذه النتائج، حيث لم تحدث أي تغيرات سلبية في التركيب الخلوي لأنسجة الكبد.

لذا نخلص لأن استخدام طحلب الاسبيرولينا بلاتينسيس بنسبة 8 جرام / كجم علف تحسن من جودة لون السمكة الذهبية و بالتالي لزيادة سعرها.

الكلمات المفتاحية: طحلب الاسبيرولينا بلاتينسيس، السمكة الذهبية، *C. auratus* و اضافات الأعلاف.