

## Article

# Enhancement of Skin Mucus Immunity, Carotenoid Content, Sexual Parameters, and Growth Response in Guppy Fish (*Poecilia reticulata*) Fed with Green Algae (*Chaetomorpha aerea*) Diets

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**Abstract:** The research aimed to analyze the influences of adding marine green algae *Chaetomorpha aerea* to the diet of guppy fish (*Poecilia reticulata*) on growth, immunological responses in skin mucus, total carotenoid content, and sexual characteristics. A total of 450 fish, with a mean body weight of  $0.19 \pm 0.1$  g and 30 fish per tank (triplicate), were randomly fed into 15 experimental tanks, each containing 50 L. Five different diets with 0, 1, 2, 4, 8, and 10% of *C. aerea* g/kg diets were fed to *P. reticulata* for 30 days. After 30 days, growth, immunological responses in skin mucus, total carotenoid content, and sexual characteristics were investigated. The results observed that the feed conversion rate and fry output were significantly ( $p > 0.05$ ) decreased in experimental groups compared to the control group. The results revealed that the dietary inclusion of *C. aerea* algal significantly increased ( $p < 0.05$ ) in mucosal immunological parameters containing lysozyme activity, myeloperoxidase activity, total immunoglobulins, and alternative complement activity, which were the highest in the group with 4% of *C. aerea* g/kg. Additionally, lateral skin and the caudal fin of fish had higher total carotenoid levels from the dietary *C. aerea* algae diet than the control group, which were the highest in the groups with 4%. Among them, 4 and 8% of *C. aerea* g/kg diet resulted in better growth performance and feed conversion ratio. Thus, the study suggested that 4% of *C. aerea* g/kg diet has enrichment of immunity, total carotenoid concentrations, and skin mucus immunity of *P. reticulata*.

**Keywords:** ornamental fish; growth performance; green algae; reproduction; skin immunity

**Key Contribution:** Marine macro algae, *C. aerea*, can be incorporated into fish diets as safe functional feeds. *C. aerea* enhanced the growth and skin mucus immune response of guppy fish. *C. aerea* regulated the sex steroid hormones and total carotenoid content levels. *C. aerea* macroalgae-enriched diet exerts an apparent immunomodulatory effect in guppy fish and thus can be suggested as a potential health-promoting fish feed supplement.



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## 1. Introduction

Ornamental fish species are produced by an aquaculture industry segment that is expanding substantially for aquarium hobbyists, which lessens the strain on declining wild stock capture rates and significantly boosts the industry's economic growth [1]. The most important tropical species traded globally is the ornamental guppy fish, *Poecilia reticulata*,

a freshwater fish native to Central America, the Caribbean, and South America. It is widespread among aquarists because of its low maintenance requirements and its appealing and variegated colors [2]. Due to water scarcity in certain regions of the world, the requirement for supervised culture conditions, including stable temperatures for tropical ornamental fish and the wish to lower the chance of disease introduction, the production of ornamental fish within closed intensive culture conditions is becoming widespread. The quality of the feed has a considerable effect on growth rate, reproductive success, and overall health since fish are raised in vast numbers on artificial feed. According to Lim et al. [3], modern techniques for ornamental fish packaging are distinguished by abnormally high fish-carrying ratios and a buildup of metabolic by-products in the transport environment after transportation. The latter is essential since ornamental fish are exported and need to be able to tolerate protracted air travel. It is well-recognized that nutritional manipulation, such as dietary supplementation with macro- and micro-elements, increases the immune response in fish, which has an impact on a fish's appropriateness for shipping. A well-known method for reducing post-shipment mortality is nutritional prophylaxis, which involves stimulating the immune systems of fish by supplementing essential nutrients to their feed [3].

The ornamental sector is impacted by the global health crisis in several ways. However, the frequency of illnesses in the agricultural industry, especially, can result in large financial losses, impeding the development of the ornamental sector [4]. A promising safe and sustainable alternative to antibiotics and vaccines is provided by medicinal plants and algae, which also boost fishes' non-specific defense immunity [5]. Ornamental fish aquaculture practices utilized a variety of herbal plants and algae for disease management and prevention of various ailments, as well as the promotion of health. In accordance with Dawood et al. [6], macro- and microalgae are safe for the environment, efficacious, quickly biodegradable, non-narcotic, non-habit-forming, and free of negative side effects. Recently, their value as a source of novel bioactive substances has grown rapidly, and researchers have revealed that marine algal-originated compounds exhibit various biological activities [7,8]. The host organism biosynthesizes these compounds as non-primary or secondary metabolites to protect themselves and maintain homeostasis in their environment; some of these secondary metabolites offer avenues for developing cost-effective, safe, and potent drugs. Those compounds already isolated from seaweeds are providing valuable ideas for the development of new drugs against cancer, microbial infections, and inflammation [9], apart from their potential ecological and industrial significance such as controlling reproduction, settlement, and biofouling, and serving as feeding deterrents [10]. Aquaculture organisms' immunity has long been studied through the use of algae meal or their extracts as a feed additive [11]. Feeds from algae have also been investigated as a likely fish feed replacement to lower the cost of making fish feed [12,13]. Sulfur-containing polysaccharides, which are only found in algal meal, are absent from terrestrial plants.

*Chaetomorpha* is a common and widespread green seaweed genus characterized by unbranched filaments [14]. *Chaetomorpha*, also known as Spaghetti algae, contains vitamins C and A [15]. Some species are edible, such as *C. crassa*, *C. linum*, and *C. brachygonia*. *C. crassa* is consumed as salad or dessert in Far Eastern countries due to its characteristic of gelatinization [16]. Apparently, there has been no study done so far on skin mucus immunity and sex steroid hormones in guppy fish of filamentous green seaweeds from India. Tsutsui et al. [17] and Sattanathan et al. [18] reported that *Chaetomorpha* sp. is an effective supplement that improves the growth performance and feed conversion rates of *Penaeus monodon* and *Labeo rohita*, respectively. While *C. antennina* had access to the methanolic extract, *L. rohita* showed increased specific and non-specific immunity [19]. Similar conclusions were drawn about how a meal including *C. linum* and *Zostera marina* affected the sea cucumber *Apostichopus japonicas*' growth and development, food consumption, and energy levels [20]. Therefore, the focal aim of this pilot study examined the effects of *C. aerea* on *P. reticulata* growth, sexual hormone levels, total carotenoids, and skin mucus immunological response.

## 2. Materials and Methods

### 2.1. Experimental Fish and Their Maintenance

Guppies in good health were obtained from Dimapur, Nagaland, after being bought from a local aquarium fish farm, and they were then kept for two weeks in a lab environment for acclimatization in tanks with 50 L capacity. Weekly, twice-syphoning with a 50% water exchange was employed throughout the trial to clean the tanks and eliminate residual feed and waste. After acclimatization, the fish, with an average body weight of  $0.19 \pm 0.1$  g, were divided into five major treatment groups for the administration of different dosages of *C. aerea* through feed. Experiments were performed in rectangular plastic tanks ( $95 \times 70 \times 60$  cm, 180 L), and dechlorinated water was used for rearing. Guppy fish ( $n = 450$ ) were distributed into 15 tanks, with each tank containing 30 (1:1 ratio of male and female fish) fish and maintained in triplicate. Water quality was monitored throughout the experiment. The temperature was  $28 \pm 2$  °C, dissolved oxygen concentration was  $5.8 \pm 0.3$  mg/L, and ammonia–nitrogen concentration was  $0.032 \pm 0.001$  mg/L during the experiment period.

### 2.2. Collection and Preparation of Seaweed

Algae of the species *C. aerea* were gathered in Parangipettai, Chidambaram, Tamil Nadu, India. After being thoroughly rinsed with tap water two or three times, the collected marine green algae were immersed in distilled water for 30 min to eliminate soil particles. After drying for seven days in the shade, they were ground. After being ground, the algal dry powder was kept chilled at 4 °C.

### 2.3. Diet Preparation and Experimental Design

The experimental composition of the basal diet was dry matter  $61.43 \pm 2.45$ ; ash content  $2.50 \pm 0.45$ ; total protein  $40.12 \pm 2.14$ ; total lipid  $3.91 \pm 1.01$ ; total fiber content  $3.50 \pm 0.87$ . Five experimental diets were prepared by incorporating *C. aerea* at concentrations of 0 (control), 1, 2, 4, and 8 g/kg feed in basal ingredients such as rice bran, soyabean meal, fish meal, corn flour, wheat flour, iodine salt, vegetable oil, and vitamin and mineral mixture (Table 1). First, dry ingredients were mixed thoroughly, and the required water was added and mixed thoroughly in a mixer for 30 min. The resulting dough was pelleted, dried at room temperature for two days, and then stored in airtight sterile containers at room temperature until feeding [18]. Feeding rate was adjusted by monitoring daily feed intake, and accordingly, each of the diets was fed to the fish in all triplicate tanks of every treatment group at a feeding rate 2% of body weight per day for 30 days. The daily ration was subdivided into two feeds at 09.00 h and 17:00 h. The institutional ethical clearance committee from St. Joseph University endorsed all the testing procedures.

**Table 1.** Composition of ingredients in experimental diets with desired crude protein and lipid levels (for 1 kg).

Ingredients	Quantity (in g/kg Diet)
Rice bran	170
Soya bean meal	550
Dry fish meal	50
Wheat flour	20
Corn flour	150
Vegetable oil ( <i>v/w</i> )	25
Iodine salt	10
Vitamin mineral mixture	25

#### 2.4. Growth Performance and Fry Production

Both at the beginning and at the end of the experiment, the fish were weighted on a digital balance accurate to 0.0001 g. Weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and fry production were calculated as follows [21].

$$\text{Weight gain (g)} = \text{Final weight} - \text{Initial weight}$$

$$\text{FCR (g)} = [\text{Feed given (Dry weight)}] / [\text{Body weight gain (Wet weight)}]$$

$$\text{SGR (\%)} = \{[\text{Ln (Final weight)}] - \text{Ln (Initial weight)}\} / \text{Total days of experiment} \times 100$$

$$\text{Fry production} = \text{Number of produced fry} / \text{Number of female fishes}$$

$$\text{Survival rate (\%)} = \text{Total fish harvested} / \text{Total fish stocked} \times 100$$

#### 2.5. Fish Skin Mucus Collection

The collection of mucus from fish skin was performed as per the methods described by Bishat et al. [21]. In this modified method, guppies ( $n = 10$ ) were anesthetized with clove oil ( $5 \text{ mL}^{-1}$ ) and placed in vicinity of 10 mL of 50 mM NaCl in individual plastic bags. Then, these were gently rubbed inside the plastic for 2 min. Mucus samples were centrifuged at  $2000 \times g$  for 8 min at  $4^\circ \text{C}$ . The supernatant was collected and stored at  $-80^\circ \text{C}$  until further analysis.

#### 2.6. Skin Mucus Immunological Parameters

##### 2.6.1. Lysozyme Activity

Lysozyme activity was measured based on the lysis of the lysozyme-sensitive gram-positive bacterium *Microbacterium lysodeikticus* (Sigma, Saint Louis, MO, USA), according to the method [22]. In summary, 25  $\mu\text{L}$  of plasma was added to 96-well ELISA plates. Then, 175  $\mu\text{L}$  of an *M. lysodeikticus* bacterial suspension was added, and optical density was measured on a spectrophotometer at 450 nm.

##### 2.6.2. Alternative Complement Activity

Alternative complement activity was determined according to the method by Yano [23] in an altered manner. Briefly, human red blood cells (HuRBCs) were collected from a volunteer. Fish mucus samples were added to Hanks balanced salt solution (HBSS), containing magnesium ion ( $1 \text{ mM Mg}^{2+}$ ), 10 mM EGTA (Ethylene glycol tetraacetic acid), and 6.7 mM HEPES (N-(2-Hydroxyethyl) piperazine-N(2-ethanesulfonic acid)). By adding 100  $\mu\text{L}$  of HuRBCs to the reaction mixture, it was maintained at  $22^\circ \text{C}$  for 90 min. The reaction was interrupted by adding HBSS containing EDTA (Etyelene glycol tetraacetic acid) and centrifuging at  $600 \times g$  rpm for 5 min. At 414 nm, the absorbance of the supernatant was measured.

##### 2.6.3. Myeloperoxidase Activity

Myeloperoxidase (MPO) content was determined by the method described by Quade and Roth [24] with slight modifications. In a sterile 96-well plate, a mixture of fish mucus (10  $\mu\text{L}$ ) mixed with 90  $\mu\text{L}$  of phenol-free HBSS, 25  $\mu\text{L}$  of 20 mM TMB (3,3', 5,5'-tetramethyl benzidine hydrochloride), and 25  $\mu\text{L}$  of 5 mM  $\text{H}_2\text{O}_2$  was taken and incubated for 15 min at  $30^\circ \text{C}$ . Later, 50  $\mu\text{L}$  of 2 mM  $\text{H}_2\text{SO}_4$  was used to stop the reaction, and the absorbance was measured at 450 nm using a plate reader.

##### 2.6.4. Total Immunoglobulin

The total immunoglobulin (Ig) concentration in plasma was determined using the Siwicki and Anderson [25] method. The technique was based on measuring total protein levels in plasma using Lowry's micro protein determination method before and after precipitating immunoglobulin molecules with a 12% polyethylene glycol solution (Sigma). The difference in protein content was used to calculate the Ig content of guppy fish.

### 2.6.5. Total Carotenoid Concentrations

The total carotenoids in fish tissue samples were determined using the procedures reported by Lee [26], with some modifications. Samples of fish skin from both lateral sides and the whole caudal fin were obtained separately. Then, 20 mL of acetone and methanol (1:1 *v/v*) was used to extract samples for 30 min. After that, the extracts were mixed with 20 mL of petroleum ether in a separation funnel. Following this, the upper phase was separated, and then one-third of distilled water was added and vacuum dried at 40 °C. Finally, the absorbance of the residue in petroleum ether was read at 450 nm using a spectrophotometer (Systronic, Digital 108, Gujarat, India).

### 2.6.6. Evaluation of Sex Steroid Hormones

Whole-body homogenate was examined for the presence of steroids at the end of the trial. All female ( $n = 15$ ) guppies were homogenized with 25 mM Tris-HCl buffer, 0.01% aprotinin, and 0.5 mM PMSF (pH 8.0), after which the supernatant was separated and kept at  $-80$  °C after being centrifuged at  $100,000 \times g$  for 1 h. The protocol described by Feist et al. [27] was used to extract sex steroids from the homogenate. Radioimmunoassay was used to measure hormone levels [28]. Samples and standard solutions totaling 40–50  $\mu$ L were added to tubes coated with mouse polyclonal antibodies. All experimental tubes were then filled with 500 l of iodine-labeled hormones (estradiol (E2), 170 kBq, 200 kBq, and progesterone (P), 185 kBq, Mimotec, Sion, Switzerland) or 1 mL of 17-hydroxyprogesterone (17-OHP), 185 kBq, (Sigma Chemicals, Saint Louis, Missouri, USA) and incubated in a water bath for 30 min at 40 °C. The level of radioactive activity was measured using a gamma counter (RX-105, Taipei, Taiwan) after washing with phosphate buffer. Estradiol had a standard concentration range of 0 to 430 nm/mL, testosterone 0 to 18.7, progesterone 0 to 71 ng/mL, and 17-hydroxyprogesterone 0 to 96 ng/mL.

### 2.7. Statistical Analysis

Each assay was repeated three times, and a statistically significant difference between the control and treatment groups was detected. The studies' triplicate data were evaluated, and the results are expressed as mean  $\pm$  standard error (SE) values with significant levels ( $p < 0.05$ ) using one-way ANOVA and the DMRT HSD test.

## 3. Results

### 3.1. Growth Parameters

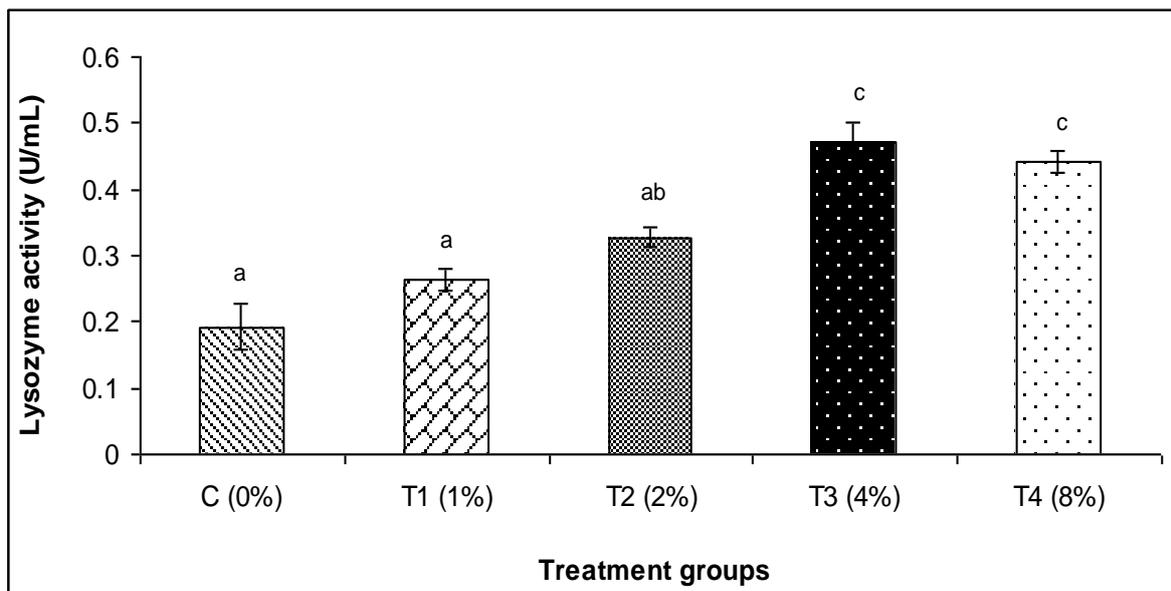
Gradual increases in the growth performance of guppy fish treated with *C. aerea* algae were observed and tabulated in Table 2. Although there was significantly decrease in fry production, there was substantial variation ( $p > 0.05$ ) in growth rate, SGR, FCR, and survival rate when utilizing the *C. aerea* algae supplemented diet, as well as significant variations in fry production between the experimental groups. No mortality was observed during the experimental study.

**Table 2.** Weight gain, specific growth rate, feed conversion rate, fry production of guppy fish fed with diets supplemented with varying levels of *C. aerea*. The values represented were the means  $\pm$  SE. Values in the same row with different superscripts are significantly different ( $p < 0.05$ ).

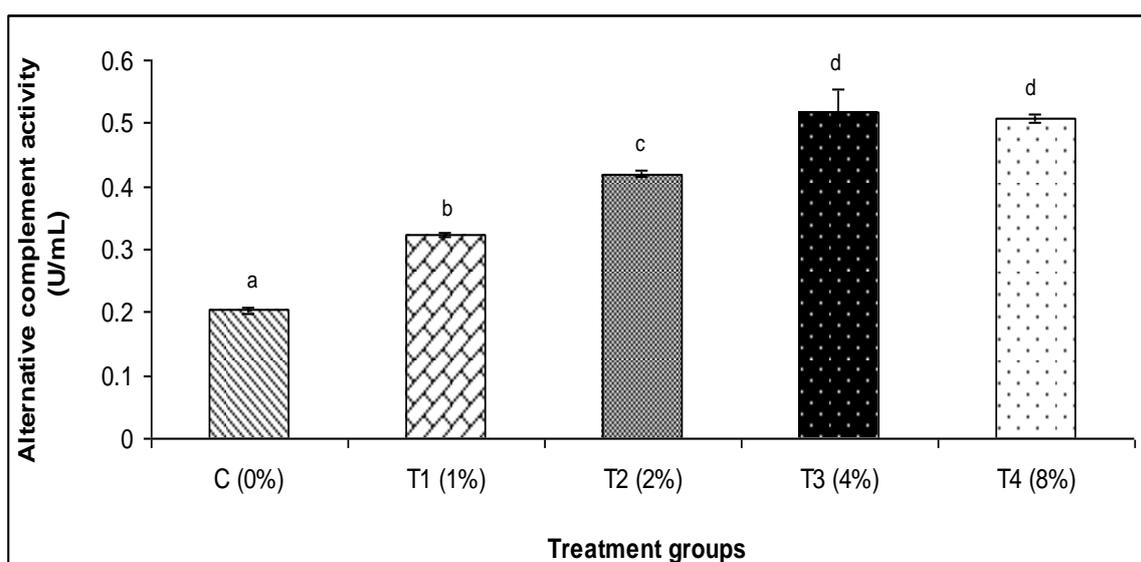
Parameters	Control	T1	T2	T3	T4
Initial weight (g)	0.019 $\pm$ 0.0006 <sup>a</sup>	0.019 $\pm$ 0.0007 <sup>a</sup>	0.019 $\pm$ 0.0005 <sup>a</sup>	0.019 $\pm$ 0.0006 <sup>a</sup>	0.019 $\pm$ 0.0003 <sup>a</sup>
Final weight (g)	0.028 $\pm$ 0.0011 <sup>a</sup>	0.031 $\pm$ 0.0006 <sup>b</sup>	0.034 $\pm$ 0.0004 <sup>c</sup>	0.040 $\pm$ 0.0008 <sup>d</sup>	0.041 $\pm$ 0.0007 <sup>d</sup>
Weight gain (g)	0.010 $\pm$ 0.0011 <sup>a</sup>	0.012 $\pm$ 0.0005 <sup>a</sup>	0.015 $\pm$ 0.0005 <sup>b</sup>	0.021 $\pm$ 0.0008 <sup>c</sup>	0.022 $\pm$ 0.0005 <sup>c</sup>
Specific growth rate (%)	0.41 $\pm$ 0.008 <sup>a</sup>	0.46 $\pm$ 0.003 <sup>b</sup>	0.58 $\pm$ 0.006 <sup>c</sup>	0.74 $\pm$ 0.006 <sup>d</sup>	0.77 $\pm$ 0.005 <sup>e</sup>
Feed conversion ratio (g)	0.077 $\pm$ 0.0009 <sup>d</sup>	0.062 $\pm$ 0.0007 <sup>c</sup>	0.050 $\pm$ 0.0003 <sup>b</sup>	0.035 $\pm$ 0.0006 <sup>a</sup>	0.034 $\pm$ 0.001 <sup>a</sup>
Fry production	0.735 $\pm$ 0.004 <sup>a</sup>	1.00 $\pm$ 0.114 <sup>b</sup>	1.55 $\pm$ 0.04 <sup>c</sup>	1.83 $\pm$ 0.060 <sup>d</sup>	2.06 $\pm$ 0.04 <sup>e</sup>
Survival rate (%)	100 $\pm$ 0.00 <sup>a</sup>				

### 3.2. Skin Mucus Immunity

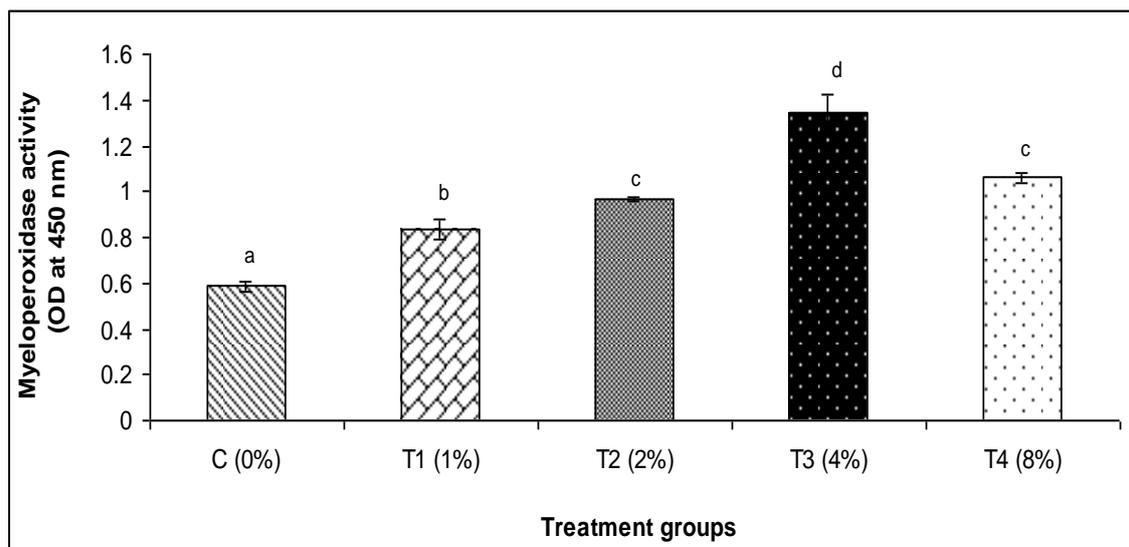
The findings of this investigation revealed that the various amounts of *C. aerea* algae greatly raised the activity of lysozyme and myeloperoxidase, alternative complement activity (ACH50), and Ig (Figures 1–5). The T3 treatment group, which received 4% more *C. aerea* algae, had the greatest level of lysozyme. With an increase in *C. aerea* algae content, lysozyme activity rose considerably ( $p < 0.05$ ). Similarly, guppy mucus from *C. aerea* algae-treated fish showed a considerable increase in alternative complement activity; the greatest value was noted in response to a 4% diet treatment. The MPO activity values in fish considerably increased ( $p < 0.05$ ), with diet concentrations of *C. aerea* algae increasing from 2 to 4%. Both the 4 and 8% treatments considerably raised the Ig content.



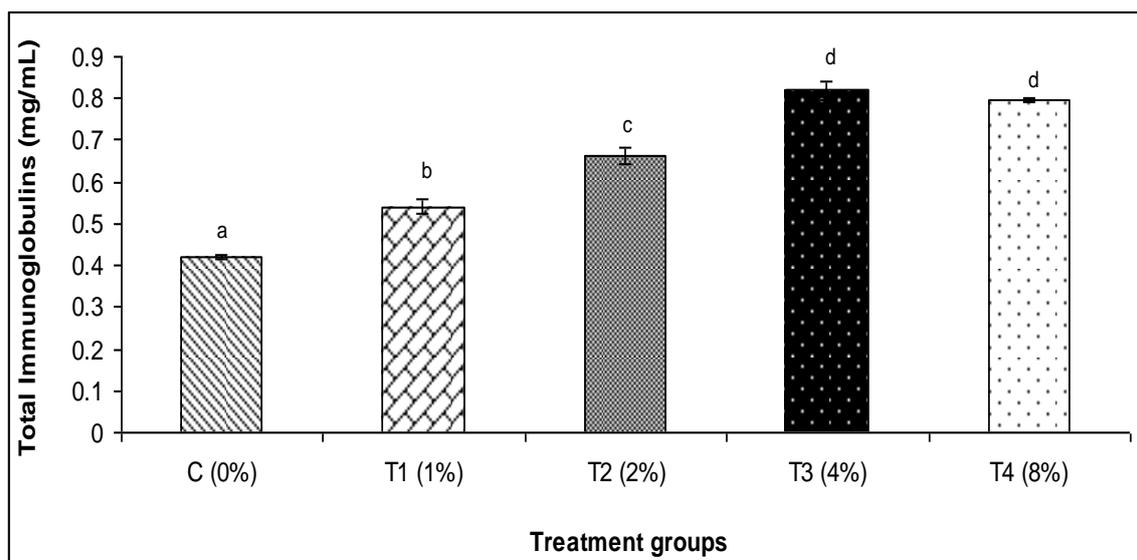
**Figure 1.** Effect of marine algae *C. aerea* on lysozyme activity of *Poecilia reticulata*. The values represented were the means  $\pm$  SE. Values in the same row with different superscripts are significantly different ( $p < 0.05$ ).



**Figure 2.** Effect of marine algae *C. aerea* on alternative complement (ACH50) activity of *Poecilia reticulata*. The values represented were the means  $\pm$  SE. Values in the same row with different superscripts are significantly different ( $p < 0.05$ ).



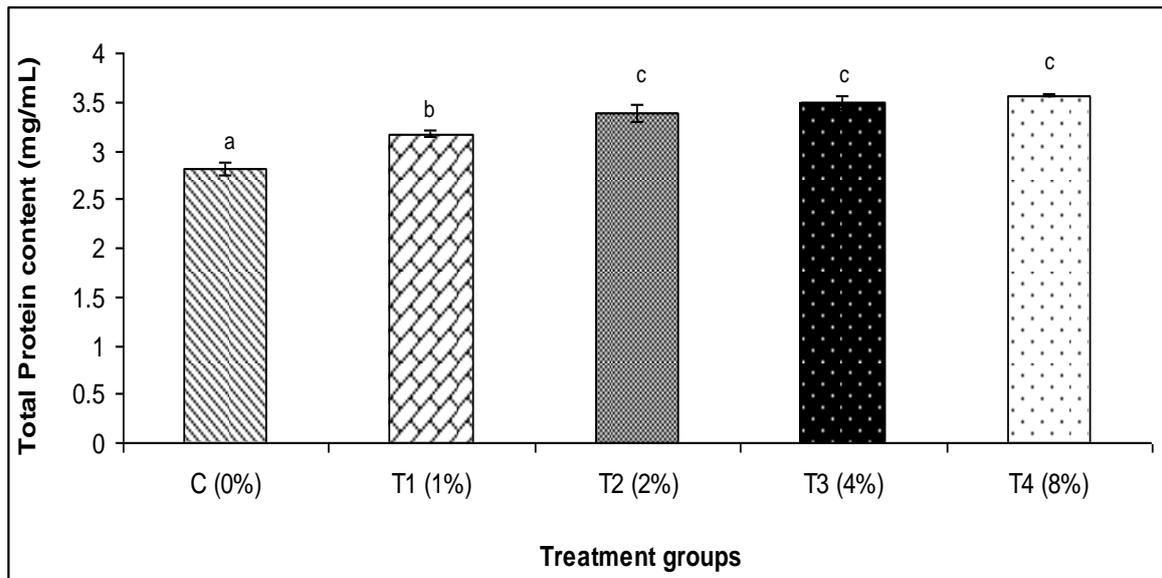
**Figure 3.** Effect of marine algae *C. aerea* on myeloperoxidase activity of *Poecilia reticulata*. The values represented were the means  $\pm$  SE. Values in the same row with different superscripts are significantly different ( $p < 0.05$ ).



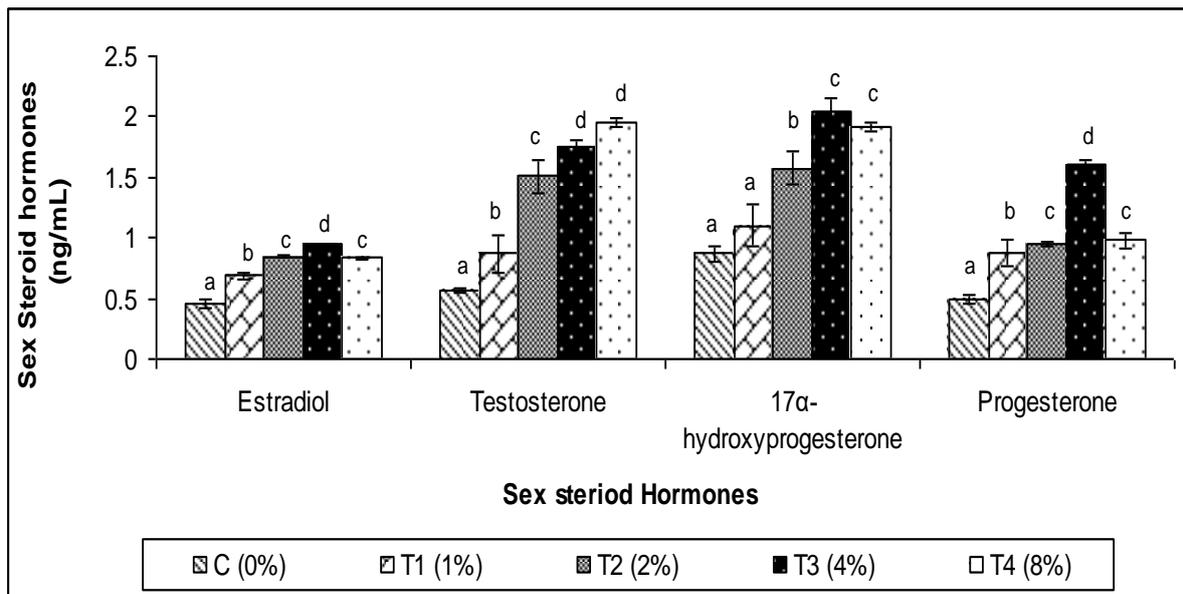
**Figure 4.** Effect of marine algae *C. aerea* on Ig levels of *Poecilia reticulata*. The values represented were the means  $\pm$  SE. Values in the same row with different superscripts are significantly different ( $p < 0.05$ ).

### 3.3. Sex Steroid Hormones

The levels of the steroid hormones (E2, T, 17-OHP, and P) in *P. reticulata* fish provided with various levels of *C. aerea* algae are shown in Figure 6. All sex steroid hormone concentrations were considerably higher ( $p < 0.05$ ) in fish provided with *C. aerea* algae. The group that received the T3 treatment had the greatest levels of sex steroid hormones.



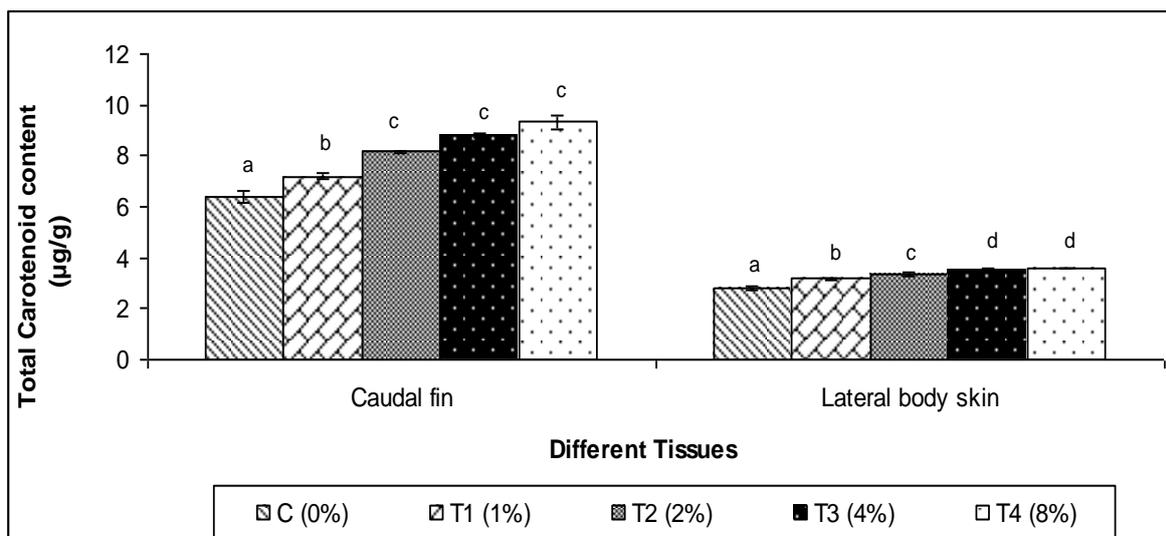
**Figure 5.** Effect of marine algae *C. aerea* on total mucus protein (mg/mL) levels of *Poecilia reticulata*. The values represented were the means  $\pm$  SE. Values in the same row with different superscripts are significantly different ( $p < 0.05$ ).



**Figure 6.** Effect of marine algae *C. aerea* on sex steroid hormone concentrations of *Poecilia reticulata*. The values represented were the means  $\pm$  SE. Values in the same row with different superscripts are significantly different ( $p < 0.05$ ).

### 3.4. Total Carotenoid Content

Additionally, total carotenoids were found in considerably higher amounts in both the skin and caudal fin of guppy fish diets including algae *C. aerea* ( $p < 0.05$ , Figure 7). Therefore, among the several treated groups, the fish that were fed 4% of *C. aerea* algae had the greatest total carotenoid contents in both tissues.



**Figure 7.** Effect of marine algae *C. aerea* on total carotenoid content in lateral skin and caudal fin of *Poecilia reticulata*. The values represented were the means  $\pm$  SE. Values in the same row with different superscripts are significantly different ( $p < 0.05$ ).

#### 4. Discussion

The increasing interest in the use of herbs for boosting growth and immunity in fish has originated recently. Many plant- and algae-derived compounds have been found to trigger both specific and non-specific immune responses in animals, which are more evaluated in finfish [29–31]. It is uncertain whether clay and marine algae are used together as an additional feed source for aquatic life. Current research has revealed that dietary supplementation with *C. aerea* of green marine algae has been researched and demonstrated to improve fish and prawn growth performance and food utilization [17,18]. However, this investigation study reveals that fish provided with *C. aerea* algae for 30 days enhanced the production of their fry, the feed conversion rate, and growth metrics (growth rate, specific growth rate) that were optimized.

Guppy growth performance in the current feeding trial significantly changed when meals containing different concentrations of *C. aerea*. According to Bishat et al. [21], guppy fish fed diets with 10–15% *Moringa oleifera* showed improved growth performance. According to Sattanathan et al. [19], *C. aerea* performed similarly to fish administration through the intra-peritoneal cavity of *C. aerea* extract in terms of various growth parameters and food utilization responses in *L. rohita*. These findings are consistent with the current research. Studies on ornamental guppies reveal that feeding garlic extract significantly modifies the growth interpretation of guppy fish [32]. The findings of Hindu et al. [33] highlighted the bioactive compounds in the algae, including minerals, polyunsaturated fatty acids, polyphenols, different pigments, and growth hormones, which appear to be the reason for the parameters' favorable impacts. *Labeo rohita*'s development and feeding effectiveness were both enhanced by the dietary inclusion of *C. aerea*'s 75 mg/g methanolic extract, according to Sattanathan et al. [34]. According to the literature mentioned above, *C. aerea*'s inclusion in the present investigation boosted the performance of growth and utilization of the feed. The benefits of medicinal plants and algae to boost fish health and immunological development have gained popularity recently. A variety of compounds produced by algae cause fish to react immunologically in both specific and non-specific ways [35]. In commercial aquaculture, green algae are employed for many different purposes, such as nutrients, growth promoters, and antibacterial agents. Investigations are also being conducted into their ability to both prevent and treat fish infections [36]. According to the data obtainable at that point, *C. aerea* supplementation significantly affected the levels of Ig, activities of MPO and lysozyme, as well as ACH50 in the diet. As the concentration of *C. aerea* algae in

the diet increased, the skin mucus immunity responses in the guppy diet increased as well, reaching their maximum values in guppies fed with 4% *C. aerea* algae concentration.

Oral administration of plant-derived bioactive compounds has been shown to impact reproductive activities, boost immunological responses, and encourage growth in aquatic animals [37–39]. Fish sex reversal, delayed maturity, and increased fertility have all been made possible by plant extracts [35,36,40,41]. Hormonal function has been demonstrated by several bioactive chemicals that have been separated from plants. The most active phytoestrogens have estrogenic activity and interact with oestrogen receptors, whereas interactions between isoflavones and phytoestrogens and the androgen and progesterone receptors are less prevalent [42,43]. Earlier studies have shown that plant compounds can mimic sex hormones as agonists or antagonists to nuclear receptors, as well as modulate the biosynthesis of sex hormones by altering the activity and expression of enzymes [44–47]. An ethanol extract was initially discovered as a possible endocrine disruptor in an investigation on reproductive efficiency and steroid hormone levels in zebrafish treated with the RE isolated from *Ruta graveolens* L. [48]. Plant extracts have been studied as possible replacements for controlling reproduction to produce monosex populations in *Tilapia* culture [37]. Marine algal biomass has health benefits beyond enriching nutrition. As a component of functional foods, they contribute to optimal health and reduced health risks or disease prevention [49]. Many proteins, oils, carbohydrates, vitamins, carotenoids, and other nutrients are found in various species of algae [50–52]. These bioactive ingredients offer a variety of health benefits to terrestrial and aquatic organisms. For instance, they protect against diseases, prevent nutrient deficiencies, and promote proper growth and development among aquaculture species [53,54]. It is possible that the rich proteins or beta-carotene may artificially enhance the color of ornamental fish.

Research on the impact of plants on the reproductive markers in farmed fish is crucial, particularly when considering long-term use, to prevent unintended detrimental consequences on the fish's capacity to reproduce. The protein content of macroalgae varies greatly from phylum to phylum [55]. Generally, the protein fraction of brown seaweeds is low (3–15% of dry weight) compared with that of green or red seaweeds (10–47% of dry weight) [56]. The protein in macroalgae contains all essential amino acids; however, variations in their concentrations are known to occur [57]. Recently, much attention has been paid to unraveling the structural, compositional, and sequential properties of bioactive peptides. Bioactive peptides usually contain 3–20 amino acid residues, and their activities are based on their amino acid composition and sequence [58,59]. These peptides are reported to be involved in various biological functions such as antihypertension, immunomodulatory, antithrombotic, antioxidant, anticancer, and antimicrobial activities, in addition to nutrient utilization [59,60]. Contrary to other studies' findings indicating that the group not receiving algae therapy had a decline in sex hormone levels, our findings demonstrated that adding *C. aerea* to meals dramatically enhanced the synthesis of sex hormones. Consuming plants and algal extracts may prevent cholesterol from being transferred to the mitochondria in Leydig cells and from being converted to testosterone, preventing the production of steroid hormones. Consuming green algae and herbal plant extracts may also harm Leydig cells and prevent the expression of enzymes involved in the steroidogenic process [61]. Despite this, our study showed that this research is the first to consider how *C. aerea* algae can affect fish reproduction. The literature lacks the presence of any additional articles on this issue. Undoubtedly, significant issues that call for further study are how these effects function biologically and whether they affect an adult's capacity for reproduction. The highest carotenoid concentrations were seen in fish that were fed 4% *C. aerea* algae. Yanar et al. [62] documented the growing impacts of sweet and hot red pepper as a food supplement for rainbow trout fish coloring. Red pepper enhances the coloration of the yellowtail cichlid's tail, as claimed [63]. Red pepper meal is a natural source of carotenoids for blue streak hap, as stated by Yimaz and Ergun [64].

Fish develop brilliant colors due to red pepper's optimal level of body tissue carotenoids, according to reports by Yilmaz et al. [65]. Additionally, it appears that the chemical compo-

nents in this herbal supplement have favorable effects on pigmentation, although studies on the benefits of garlic or onion feed on the pigmentation of fish have not been discovered. For instance, anthocyanin comprises 10% of red onions' flavonoid concentration [66]. These are naturally occurring plant pigments that give plants and some vegetables their different purple colors (red, blue, and purple). According to the study by Wang et al. [67], allicin isolated from garlic is a poorly understood oxygenated carotenoid. The flavonoids and carotenoids in the herbal additives used in this investigation therefore seem to produce higher coloration than the control group.

Carotenoids are synthesized from geranyldiphosphate by all photosynthetic organisms [68]. Macro- and microalgae, which are important in the production of larval fish because of their nutritive ingredients, can be used as a natural pigment source in fish feeds. The use of algal biomass has been recently investigated regarding its potential as a coloring agent [69,70]. However, the use of synthetic pigment sources is more common because they are easy to obtain [71]. There is no study on the effect of natural and synthetic pigments on the color of guppy fish. Skin coloration is one of the most important marketing criteria in the ornamental fish trade [72]. Dietary additives such as essential fatty acids, alpha-tocopherol, ascorbic acid, and carotenoids influence the reproductive performance and coloring of fish [72]. Enhanced growth performance, health, nutritional performance, and coloration have been declared with dietary small amounts of microalgae inclusion (1–5%) in a range of fish species [72,73]. *Spirulina* is a microalga commercially used as a supplementary food in human nutrition and finfish diets due to its rich source of protein, indispensable fatty acids, essential amino acids, vitamins, and minerals [72,74–76]. The effects of dietary *Spirulina* on the growth performance and skin coloration have been studied for ornamental finfish species, including red swordtail, *Xiphophorus helleri* [77], goldfish, *Carassius auratus* [78,79], yellow tail cichlid, *Pseudo tropheusacei* [72], and three-spot gourami, *Trichopodus trichopterus* [80].

## 5. Conclusions

In conclusion, feeding guppy fish with *C. aerea*, the only feed that contains two protein sources as well as macro green algal and pigment additives, resulted in an average final weight but also in the highest skin mucosal immunity and body pigments compared to the control group. Additionally, feeding guppy fish with *C. aerea* algae improved growth, fry generation, and survival rate. The overall findings of this study showed that *P. reticulata* fry's production and the concentration of sex hormone levels were enhanced by the mean optimal dosage of *C. aerea* algae-supplemented food.

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