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# Seaweed *Ulva reticulata* a Potential Feed Supplement for Growth, Colouration and Disease Resistance in Fresh Water Ornamental Gold Fish, *Carassius auratus*

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## Abstract

Gold fish (*Carassius auratus*) being an omnivorous fish was chosen as the candidate fish. Fingerling of *C. auratus* 2.34 gm after acclimated to laboratory condition for a period of 15 days were used in various studies. Five feeding types with 35% dietary protein level and with varying levels of algal meal were prepared and used. The fish were divided in to 5 groups. Group I was fed on *Ulva reticulata* free diet. Fish belonging to groups II, III, IV and V were fed on diets with 2, 4, 6 and 8% of *Ulva reticulata* supplementation respectively for 40 days. At the end of the experimental period growth, proximate composition, haematological parameters and carotenoid content were analyzed. It can be conclusively deduced from this study that *Ulva reticulata* had positively improved growth performance, haematological parameters and coloration in gold fish *C. auratus*. Reasons could be the high nutrient and mineral profile and carotene and chlorophyll a, b content of *Ulva reticulata*. This study suggests that *U. reticulata* can be included as an ingredient of gold fish diet up to 8%. Owing to the presence of disease resistance imparting antibacterial substances in the algae gold fish remained active and healthy and were free from bacterial infection, during the period of investigation. Antibacterial activity of *U. reticulata* on the tested fish pathogens indicates the scope of using extracts of *U. reticulata* an immuno- prophylactic for health management in the culture of Gold fish. Based on the results of this study, it was concluded that short term feeding had no adverse effect on gold fish but long term effect needs future research. Future work should focus on digestibility coefficients of different nutrient classes in *U. reticulata* prior to inclusion in formulated diets.

**Keywords:** Goldfish; Seaweed; *Ulva reticulate*; *Carassius auratus*; Supplement feed

## Introduction

Ornamental fishes can be defined as attractive, colorful fishes of peaceful nature that are kept as pets in confined spaces of an aquarium or a garden pool as visually exciting objects [1]. However, some fish species loved by aquarists are quite ugly, in such cases the peculiar appearance is a source of attraction for the aquarium lovers and naturalists [2]. Inspiring popularity of aquarium keeping in households in many parts of the world, ornamental fish has become an important part in international trade. The world trade of ornamental fish is valued at about US $ 9.0 billion [3].

In Indian domestic trade in ornamental fish is conservatively estimated at Rs.10 crores and it is growing at the rate of 20 percent annually and the present domestic demand is higher than the supply [4]. *Carassius auratus* is a traditional ornamental gold fish which the body and fin shape; size and colour are features that affect its market price [5,6]. Attractive coloration is one of the most important quality criteria dictating their market value [7]. Carotenoids are the primary source of pigmentation on the skin of fishes. Like other animals, fish are unable to perform *de novo* synthesis of carotenoid [8]. In natural environment the fishes meet their carotenoid requirements by ingesting aquatic plants or through their food chain [9].

Skin colour determination in the gold fish involves cellular factors like existence of cells called choromatophores which store specific pigment black or brown (melanophores), yellow or orange (xanthophores), red (Erythrophores), iridescent, blue, silver or gold (iridophores) and white (leucophores) [10]. However, pigmentation efficiency is associated with different factors such as pigment source, fish size, feeding time, diet composition, sexual maturity and genetics of organisms [5,11]. Haematological parameters have been recognized as valuable tools for monitoring fish health. The analysis of blood indices has proven to be a valuable approach for analyzing the health status of farmed animal as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting [12].

The immunity stimulating capacity of algae *Spirulina* and *Cladophora* have well been established [13,14]. Algae meals are an alternative plant feed stuffs that are increasingly being used in aqua feeds because of their nutritional quality, lower cost and availability [15,16]. Fish meal representing the protein source, is the main ingredient in fish feed. Up to 1990, about 10% of the global fish meal production was used for aquaculture; however this has tripled over the last decade [17]. The addition of small amounts of algae meal to fish diets resulted in considerable effects on growth, feed utilization [18,19]. *Ulva* species have become important macro algae, as it contains proteins, pigments, vitamins, minerals and unknown growth factors for several cultivable food fishes which have been investigated as a dietary ingredient including black sea bream, *Acanthopigrus schelegli*

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[18], snake head, *Channa striatus* [20], red sea bream, *Pagrus major* [21] and striped mullet, *Mugil cephalus* [22,23] for a wide range of cultivable food fishes. Low level dietary incorporation of *Ulva* meal has resulted in improved growth, feed utilization, physiological activity, disease resistance, carcass quality and reduced stress response.

The gold fish is an omnivorous fish and can utilize feed including plant materials effectively. Seaweeds are recognized as a potential source of bioactive natural products [24]. They are considered as a source of secondary metabolites characterized by a spectrum of biological activity. Seaweeds are rich and varied source of biocidal and pharmaceutical agents. Recent findings evidenced that seaweeds contained antibacterial [25], antiviral [26,27], antifungal, Cytotoxic [28] and larvicidal potential [29]. In aquaculture the bacterial pathogens create with fish diseases is worldwide problem. The secondary metabolite directly acts as antibiotics in aqua culture. Hence, the interest in macro algae as a potential and promising source of pharmaceutical agents has increased during the last years [30,31]. Extracts from seaweeds have let to the discovery of the variety of secondary metabolites with antimicrobial activities against fish pathogens.

The present study was carried out to examine the efficiency of seaweed *Ulva reticulata* as a feed ingredient for gold fish *Carassius auratus*. Evaluate growth and coloration of *Carassius auratus* fed on diets with various level of *Ulva reticulata* meals as feed additives so as to determine the optimal level of dietary supplementation. Estimate the proximate chemical composition from fish *Carassius auratus*. Access the haematological parameters of *Carassius auratus* fed feed with the varying level of *Ulva* meal. Determine the antimicrobial efficacy of *Ulva reticulata* on some selected bacterial fish pathogens viz., *Pseudomonas aeruginosa and Aeromonas hydrophila.*

## Materials and Methods

### Sample collection

Fresh water ornamental fishes were collected from ornamental fish trades, Chennai and they were accumulated with the laboratory condition. Raw samples of seaweed were collected from Hare Island, Thoothukudi, Tamilnadu.

### Experimental species

The gold fish is one of the earliest fish to be domesticated, and the most commonly known aquarium fish. Among ornamental fish, gold fish is considered to be the most popular and attractive pet fish due to its many variations such as colour, finagles, tail, shape, size and body and has great demand both in domestic as well as international market. The gold fish is an omnivorous fish and can utilize feed including plant materials effectively. The ornamental fish *Carassius auratus* (Figure 1a) was chosen as the candidate fish for the present study. It belongs to Order-Cypriniformes, Family-Cyprinidae, Genus-*Carassius* and Species-*C. auratus.*

### Fish collection and maintenance

Full orange colour gold fish of more or less uniform size (2.75 ± 0.09 cm in length and 2.89 ± 0.02 cm in weight) were procured from a private fish farm Thirunavukarasu aquarium, near Korampallam, Thoothukudi, Tamil Nadu, India. They were transported to the laboratory in an oxygenated bag and stocked in a trough. The water was changed on alternative days and the fish were fed with a balanced fish diet with 35% protein. The excess feed and fecal materials were siphoned out once a day. The temperature of the water in the fish trough was kept

**Figure 1a:** Experimental fish-*Carassius auratus*.

**Figure 1b:** Experimental set-up.

between 28-29°C. Other physiochemical parameters of water were also analyzed systematically at 7 days interval to maintain optimum level of dissolved oxygen 6.8-7.2 mg/l. pH 7.7-8.5 and ammonia 0.08-0.12 mg/l throughout the experiment.

### Preparation of seaweed meal

The seaweed *Ulva reticulata* was collected from the coastal areas of Tuticorin. This species was chosen because of its abundance, distribution and proximity to areas and seaweed also chosen because it is known as one of the species that give good colour. The collected samples were thoroughly washed with sea water, dried at 40°C for 48 to 72 hrs and then ground to powder.

### Diet formulation

In the present experiments 35% protein was used as basal diet for *Ulva* meal supplementation. Fresh feed ingredients were procured in dry form. Using the ingredients such as dried fish meal, ground nut oil cake, rice bran, wheat flour, sardine oil, vitamin and mineral mixtures the feed was formulated following the square method of food compounding [32]. The ingredients were mixed in five different proportions in order to get five diets with five levels of algae *Ulva reticulata* (0, 2, 4, 6 and 8%) in the diet. The ingredient were mixed and homogenized to wet dough by adding sufficient water. The dough was cooked under steam in a pressure cooker, cooled and to this feed vitamin mix, cod liver oil and *Ulva* meal were added and mixed then pelletized through hand pelletizer until between 1 and 4 mm and dried in shade to reduce the moisture content of the feed below 10% and stored in air tight container and used for experiment. Composition of the feed is given in Table 1.

| Ingredient (g) | Diet (Ulva Content) | | | | |
|---|---|---|---|---|---|
| | F1<br>0 | F2<br>2% | F3<br>4% | F4<br>6% | F5<br>8% |
| Fish meal | 40 | 40 | 40 | 40 | 40 |
| Ground nut oil cake | 36 | 36 | 36 | 36 | 36 |
| Tapioca flour | 14 | 13 | 12 | 11 | 10 |
| Wheat flour | 8 | 7 | 6 | 5 | 4 |
| Sardine oil | 1 | 1 | 1 | 1 | 1 |
| Vitamin & Mineral mix | 1 | 1 | 1 | 1 | 1 |
| Ulva meal | 0 | 2 | 4 | 6 | 8 |
| Proximate composition (%) | | | | | |
| Moisture | 7.27 ± 0.13 | 7.51 ± 0.05 | 7.74 ± 0.08 | 7.86 ± 0.08 | 7.89 ± 0.02 |
| Crude protein | 34.13 ± 0.17 | 34.84 ± 0.3 | 34.92 ± 0.14 | 34.92 ± 0.14 | 35.19 ± 0.7 |
| Crude fat | 6.11 ± 0.06 | 5.83 ± 0.09 | 5.69 ± 0.17 | 5.12 ± 0.23 | 4.78 ± 0.08 |
| Ash | 9.26 ± 0.06 | 10.94 ± 0.09 | 11.54 ± 0.10 | 11.69 ± 0.08 | 14.02 ± 0.06 |
| Nitrogen Free Extract | 43.22 ± 0.27 | 41.45 ± 0.14 | 40.35 ± 0.23 | 39.82 ± 0.34 | 39.96 ± 0.35 |

**Table 1:** Formulation and composition of the experimental diets and their proximate composition (% dry matter basis).

## Proximate composition analysis of feed

Proximate analysis is usually the first step in the chemical evolution of a feed ingredient, where the materials is subjected to a series of relatively simple chemical tests so as to determine the content of moisture crude protein, crude fiber, lipid, ash and digestible carbohydrate.

**Estimation of protein from formulated feed:** The protein content of the feed was estimated following the method of Lowry [33]. 100 mg of tissue was taken and homogenized with 5 ml of 10% trichloroacetic acid in a tissue homogenizer. The homogenates were centrifuged for 15 minutes at 3000 rpm. The supernatant was discarded. The precipitate was dissolved thoroughly in 5 ml of 0.1N sodium hydroxide solution and kept in a water bath at 60-70°C for 10 minutes. From this 0.5 ml of solution was pipetted out and poured in a clean dry test tube.

To this 4 ml of carbonate copper solution was added. It was mixed well by thorough lateral shaking and kept in the room temperature for 15 minutes. To this 0.4 ml of folin phenol reagent was added. The test tubes were shaken well for uniform mixing and kept in the room temperature for another 30 minutes. The blue colour appeared was read at 640 nm against a reagent blank. The standard curve was obtained by using bovine serum albumin.

**Estimation of lipid from formulated feed:** Lipid was estimated following the method of Bragdon [34]. A known quantity of the tissue was homogenized well with 5 ml of chloroform and the solution was centrifuged at 3000 rpm for 15 min. The supernatant was evaporated to dryness by keeping it in an oven. Then 3 ml of 2% potassium dichromate in conc. Sulphuric acid was added followed by 3 ml of distilled water. The greenish brown colour appeared was read at 640 nm against a reagent blank in a spectrophotometer. The standard curve was obtained by using cholesterol and the lipid was expressed in mg/g.

**Determination of Ash:** Ash content was estimated by the method of Lovell [35]. A weighed amount of dry sample was heated to higher temperature (550-600°C). All the organic constituents are burnt leaving only the inorganic constituents in the form of ash. The percentage of ash content was estimated as per the calculation given below.

On moisture free basic/Per cent by weight=$100(W_2\text{-}W)/W_1\text{-}W$

Where,

$W_2$=Weight in g of dish with ash

W=Weight in g of empty dish

$W_1$=Weight in g of the dish with the dried material taken for the determination of ash.

## Experimental design

Active and healthy fish (2.79 ± 0.15 g) were chosen from the acclimation tank and starved for 24hrs prior to the commencement of the experiment. The fish were divided into 5 groups of 6 individuals each and reared in circular plastic troughs containing 20 L of water (Figure 1b).

Group 1 served as control and fed with *Ulva* free diet ($F_1$). Test animals belonging to 2$^{nd}$, 3$^{rd}$, 4$^{th}$ and 5$^{th}$ groups were fed on diet ($F_2$, $F_3$, $F_4$ and $F_5$) with varying *Ulva* levels viz., 2, 4, 6 and 8% respectively. Fish were fed on allotted experimental diet at 5% of their total body weight per day. Feeding was done daily at 0800hrs. The unfed was removed after 2 h of feeding.

Triplicates were maintained for each group. The experiment has run for 40 days. The following experimental parameters were maintained throughout the experiment; temperature 26 ± 2°C; pH 7.7, dissolved oxygen 8 mg/l; photoperiod 12LL:12DD.

**Growth parameters:** At the beginning of the experiment, the total wet weight of the fish in each group was determined by weighing in an electronic balance. All fish in each group were weighed at the end of the experiment.

Absolute growth rate (g)=Final weight–Initial weight

Specific growth rate (%)=[(In final weight–In initial weight)/ Rearing period (days)]×100

Weight gain (mg/g)=[(Final weight–Initial weight)/Initial 0weight]×100

**Carotenoid estimation**

**Pigment extraction in fish tissue:** Analysis of total carotenoids in the fish tissue was carried out prior to and after the termination of the experiments following [36]. One gram of entire gold fish body tissue (without head and alimentary canal) was taken in a 10 ml screw capped clear glass vials and 2.5 g of anhydrous sodium sulphate was added.

The sample was gently meshed with a glass rod against the side of the vial and then 5 ml of chloroform was added and left overnight at

0°C. When the chloroform formed a clear 1-2 cm layer above the caked residue, the optical density was read at 380, 450, 470 and 500 nm, in a spectrophotometer taking 0.3 ml aliquots of chloroform diluted to 3 ml with absolute ethanol. A blank prepared in a similar manner was used for comparison. The wavelength, at maximum absorption, was used for the calculation.

The total carotenoid content was calculated as µg per wet weight of tissue as follows:

Total carotenoid content=[Absorption at maximum wave length/(0.25×sample weight (g))]×10 (Where, 10=Dilution factor; 0.25=Extinction coefficient)

## Proximate composition analysis

Analysis of proximate composition of muscle tissue of fish from all groups was made both at the beginning and at the end of the experiment.

**Estimation of protein:** The protein content of the feed was estimated following the method Raymont [37]. About 20 mg of dried material was taken and homogenized with 1.0 ml of distilled water. 4.0 ml of Biuret reagent was added into two installments of 2.0 ml each and the tissue grinder was cleaned before being transferred to centrifuged tube. After 30 minute and this sample were centrifuged for 10 minutes and the supernatant fluid was transferred to another tube. Then the optical density of supernatant tube in spectrophotometry was taken using 540 ml against blank reading. Bovine serum albumin was used as standard protein. Optical density is equal to 18.44 mg of protein. The percentage of protein was calculated using the formula.

Percentage of protein=18.44×O.D of the sample X 100/Weight of tissue

**Estimation of carbohydrate:** The Carbohydrate content of the feed was estimated following the method Seifter [38]. A known weight of tissue 10 mg was homogenized with 2 ml of 10% TCA and 8 ml distilled water. The homogenate was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected. One ml of the supernatant was taken in a clean test tube. To this 5 ml of anthrone reagent was kept in boiling water bath for 10 minutes. The tube was then cooled at room temperature. The developed colour was read at 620 nm against a reagent blank in a spectrophotometer. Standard curve was obtained by using glucose and the carbohydrate was expressed in mg/g. The values were then converted into percentage.

Percentage of Carbohydrate=2.16×O.D of the sample X 100/ Weight of tissue

**Estimation of lipid:** The Lipid content of the feed was estimated following the method Bragdon [34]. The same procedure was followed as above.

## Hematological studies

**Blood sampling:** Blood sample was collected from fish of each group. The fishes were collected and gently wiped with a dry cloth to remove water. Caudal peduncle was cut with a sharp blade and the blood was collected in a watch glass containing EDTA, an anticoagulant (6% Ethylene Diamine Tetra Acetic Acid). The blood was mixed well with the EDTA solution by using a needle and this sample was used for determining the RBC, total WBC and haemoglobin content.

**RBC count:** For RBC count, a method devised by Yokayama [39] and later modified by Christensen [40] was followed. The standard RBC diluting pipette and a 1:200 dilution was used for the RBC count. Hayem's solution was used for dilution.

Blood was drawn in the pipette up to the 0.5 mark. The tip of the pipette was wiped with a filter paper to adjust the volume exactly to 0.5 marks. The pipette was immediately filled to the 101 mark with the diluting fluid. Partial rotation of the pipette while it was being filled assured the complete mixing its ends grasped between the thumbs and diluting fluid and prevented clotting. With its ends grasped between the thumb and second finger the pipette was then shaken for 5 minutes, which ensured the through mixing of the blood with diluting fluid.

The improved Neubauer counting chamber was used for counting the cells. A glass cover slip was placed over the 'H' groove on the chamber. Then the blood was allowed to enter the chamber by simply touched to the edge between the cover slip and the chamber, where the blood spread under the cover slip by capillary action. The counting area is divided into 25 squares of 16 small squares. Only 5 squares were taken to count RBC marked as $E_1$-$E_5$. Two minutes time was giving for the corpuscles to settle down, the total number of cells in the five squares were counted, calculated and expressed as RBC per $mm^3$ of blood.

**Haemoglobin content:** The Sahli Hellige method was followed for haemoglobin determination. Sahlis pipette was filled slightly above the 20 mm mark the pipette was wiped with a filter paper or cotton to remove excess blood and the volume was adjusted to exactly 20 $mm^3$ by blotting the tip. The blood was expelled into a calibrated (transmission) test tube containing 2 ml of 0.1N HCL. The pipette was rinsed several times in the acid solution. The sample was allowed to stand for 15 minutes.

The principle behind the method is the conversion of haemoglobin to acid haematin. The acid haematin was then diluted with distilled water till the colour matched with colour of the standard in haemoglobinometer. The height of the column at which the match obtained gives the value of haemoglobin in g%.

## Determination of antibacterial activity of *ulva reticulata* against bacterial pathogens

The antibacterial activity of extract of seaweed *Ulva reticulata* was investigated against select bacterial fish pathogens such as Gram +ve (*Aeromonous hydrophila*) and Gram -ve (*Pseudomonas aeruginosa*).

**Preparation of seaweed extracts:** The collected *Ulva reticulata* samples were cleaned and the necrotic parts were removed. The seaweeds were washed with tap water to remove any associated debris and shade dried at room temperature (28 ± 2°C) for 5-8 days or until they are brittle easily by hand. After completely drying, the seaweed materials (1.0 kg) were ground to a fine powder using electrical blender. Twenty gram of powdered seaweed was extracted successively with 200 ml of solvents (Methanol, and Ethyl acetate) in Soxhelet extractor until the extract was clear. The extracts were evaporated to dryness and reduced pressure using rotary vacuum evaporator and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use.

**Preparation of algal disc for antibacterial activity:** 5 mm diameter discs were prepared using sterile Whatmann No.1 filter paper. The solvent such as (Methanol, and Ethyl acetate) extracts of seaweed *Ulva reticulata* were mixed with 1ml of Dimethyl sulfoxide (DMSO). The discs were impregnated with 20 µl of different solvent extracts of *Ulva reticulata* at three different concentrations ranging 1 mg/ml, 10 mg/ml and 100 mg/ml to check their antibacterial activity. The streptomycin (5 mg/ml) was used as positive control.

**Bacterial inoculum preparation:** Bacterial inoculums was prepared by inoculating a loopful of test organisms (*Pseudomonas aeruginosa and Aeromonas hydrophila)* in 5 ml of Nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farl and standards and then used for the determination of antibacterial activity.

**Disc diffusion method:** The antibacterial activities of *Ulva reticulata* extract were determined by Disc Diffusion method proposed by Bauer [41]. Petri plates were prepared by pouring 20 ml of Nutrient agar and allowed to solidify for the use in susceptibility test against bacteria. Plates were dried and 0.1 ml of standardized inoculum suspension was poured and uniformly spread. The excess inoculum was drained and the plates were allowed to drying for five minutes. After drying the discs with extract were placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the agar surface. The streptomycin (5 mg/disc) was used as positive control. The plates were incubated at 37°C for 24 hours. The zone of inhibition was observed and measured in millimeters.

## Statistical analysis

The statistical analysis of the data was performed as per the method described by Snedecore and Cochran [42]. Mean, Standard Deviation and Analysis of variance was analysed.

**Analysis of variance (ANOVA):** Variance between different means of a single variable was tested following one way calculation of variance described by Zar [43]. Sum of X for all the values was squared and a correction factor 'C' was obtained.

$$C = \frac{\left(\sum X\right)^2}{N}$$

Total sum of squares=$\left(\sum X\right)^2 - C$

Where, $X^2$ represents the sum of squared values.

Group sum of squares=$\frac{\left(\sum X\right)^2 - C}{N}$

Where, N- number of observations in each group.

Error sum of square=Total SS- Group SS

Considering the degrees of freedom for each source of variance, mean square was calculated.

Total SS=Number of values in the Table–1

Group SS=Number of groups–1

Error SS=Df of total SS–df group SS

Significance level at the corresponding df was read from table D.11 of Zar [40].

## Results

## Growth

All diets were readily accepted by fish, indicating no issues relating to palatability of algal supplemented diets. No mortalities were observed during the experimental period. Data indicates that dietary *Ulva* meal, significantly improved fish growth when compared with the non *Ulva* meal supplemented group. (Table 2). The mean weight of the gold fish on the first day of stocking in the control feed ($F_1$) was 2.34 gm. The initial mean weight of the fish fed on diets $F_2$, $F_3$, $F_4$ and $F_5$ were 2.52, 2.79, 2.49 and 2.44 gm respectively.

| Feed | AGR gm | SGR % | WG Mg/g % |
|---|---|---|---|
| Control | 3.01 ± 0.22 | 7.53 ± 0.55 | 128.47 ± 9.45 |
| 2% | 4.06 ± 0.72 | 11.54 ± 1.82 | 183.28 ± 29.01 |
| 4% | 4.66 ± 0.42 | 11.66 ± 1.06 | 167.05 ± 15.11 |
| 6% | 5.29 ± 0.14 | 13.24 ± 0.35 | 214.75 ± 5.72 |
| 8% | 5.68 ± 0.26 | 14.09 ± .65 | 231.24 ± 10.71 |

**Table 2:** Dietary supplementation of *Ulva reticulata* on growth parameters of *Carassius auratus*. Values are mean ± SD of 3 replicates.

| Absolute Growth Rate | | | | | |
|---|---|---|---|---|---|
| **Source of variation** | **SS** | **DF** | **MS** | **F** | **F Crit** |
| Between group<br>Within group | 12.22<br>2.44 | 4<br>10 | 3.06<br>1 | 3.06 | 3.48 |
| TOTAL | 14.66 | 14 | | | |
| **Specific Growth Rate** | | | | | |
| **Source of variation** | **SS** | **DF** | **MS** | **F** | **F Crit** |
| Between group<br>Within group | 76.38<br>9.59 | 4<br>10 | 19.1<br>0.96 | 19.9 | 3.48 |
| TOTAL | 85.97 | 14 | | | |
| **Weight Gain** | | | | | |
| **Source of variation** | **SS** | **DF** | **MS** | **F** | **F Crit** |
| Between group<br>Within group | 20299.85<br>1780.02 | 4<br>10 | 5074.96<br>178 | 28.51 | 3.48 |
| TOTAL | 22079.87 | 14 | | | |

**Table 3:** Summary of analysis of variance for the data on the feeding parameters of *Carassius auratus* in relation to dietary *Ulva* supplementation.

| Feed | Protein | | Carbohydrate | | Lipid | |
|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Initial | Final |
| F1 | 8.30% | 17.52% | 3.75% | 10.15% | 4.6% | 3.6% |
| F2 | 8.28% | 24.89% | 3.54% | 9.07% | 4.12% | 3.6% |
| F3 | 8.52% | 31.35% | 3.10% | 8.21% | 4.16% | 3.5% |
| F4 | 8.36% | 32.37% | 3.46% | 6.48% | 4.0% | 3.4% |
| F5 | 8.96% | 35.04% | 3.24% | 5.83% | 4.2% | 3.4% |

*F1- Control, F2-2%, F3-4%, F4-6%, F5-8%.

**Table 4:** Proximate Composition of muscle tissue of gold fish fed with *Ulva* meal.

The result indicates that mean weight gain of the fishes fed with diets $F_2$, $F_3$, $F_4$ and $F_5$ were found to be 4.62, 4.66, 5.29 and 5.63 gm. The absolute, specific growth rate and weight gain of the fishes also showed a similar trend of an increase with increase in concentration of *Ulva* meal in diet. The maximum values of absolute, specific growth rate and weight gain were observed in fish fed on 8% *Ulva* meal supplemented diet viz., 5.68 ± 0.26 gm, 14.09 + 0.65% and 231.24 ± 10.71 mg/g% respectively. Analysis of variance for the data on absolute growth rate, specific growth rate and weight gain indicated that the changes were significant at 1% level (Table 3).

## Body composition

Whole body composition of the fish at the beginning and at the end of the experiment is summarized in Table 4. The crude protein content of fish fed on algal diets increased proportionally with the concentration of algal meal compared to the initial measurement. Maximum tissue protein of 35.04% was realized in group fed on 8% algal supplemented diet (Figure 1a). In the present study compared with non-*Ulva* supplemented groups, fish fed *Ulva* meal resulted in a significant reduction of body lipid. The lipid level tented to decrease with increasing level of *Ulva* meal. In group fed on 8% algal diet

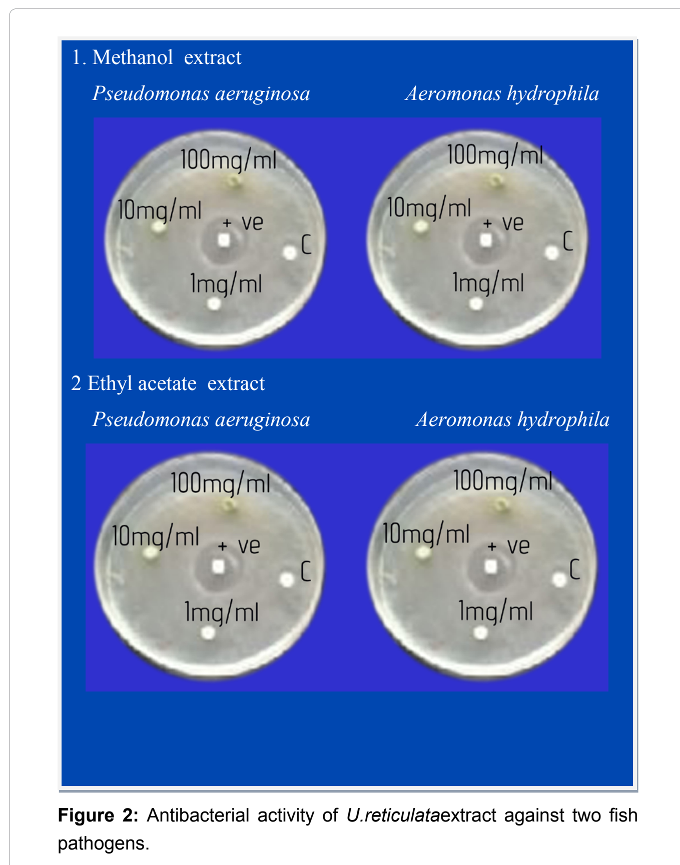


**Figure 2:** Antibacterial activity of *U.reticulata*extract against two fish pathogens.

exhibition a decrease in lipid content from the initial value of 4.2% to 3.4%.

## Coloration

The result clearly showed that the maximum carotenoid content was present in the fish fed with $F_5$ diet (8 g/100 g) dry weight feed. For instance, the total carotenoid content in the 8 per cent fed group was found (Figure 3) to be 0.60 ± 0.07 µg/g wet weight and for the control group it was found to be 0.42 ± 0.07 µg/g wet weight. Similarly, the carotenoid content in $F_2$, $F_3$, $F_4$, $F_5$ diet groups increased significantly and analysis of variance showed that the carotene content between treatments differed significantly. The carotenoid content among the variation was found to be significant (0.001) (Tables 5 and 6).

## Antibacterial activity

The results of antibacterial activities of the seaweed are summarized in Table 7. In our observation highest inhibition zone 10 mm was recorded in methanol extract (100 mg/ml) and ethyl acetate extract 7 mm of *Ulva reticulata* against *Pseudomonous aerogenosa*. The *Aeromonas hydrophila* did not show exhibitory activity against tested seaweed extracts. The positive control streptomycin was effective against all the tested bacterial pathogen and it exhibited nearly 20 mm of zone of inhibition in all the pathogens. During the entire period of investigation the fish remained active and healthy. There was no incident of any bacterial infection.

# Discussion

Natural resources as possible feed ingredient or additives need to be investigated for the development of inexpensive feeds in the world. Macro and micro algae, seaweed and other alternative aquatic plants have been researched and used for decades in aquaculture. *Ulva* meal is one of the most studied and promising source in this sense. In this study, we primarily laid out the possibility of inclusion of *Ulva* meal in gold fish *Carassius auratus* without affecting their health status negatively.

In the present study, growth performance of fish tended to increase with an increase in *Ulva* meal concentration. The growth rates recorded in the present investigation were in accordance with the finding of Mensi et al., who fed *Orechromis niloticus* fry on 30% dietary protein containing 3% and 18% *Ulva* meal for 45 days [44]. Betoikut Gorey, reported an enhancement in growth rate of *Oreochromis niloticus* fed 5-10% *Ulva* supplemented diet [45]. Whereas, Kissil observed no effect of *Ulva* inclusion on fish growth in the gift head sea bream, *Sparus aurata* [46].

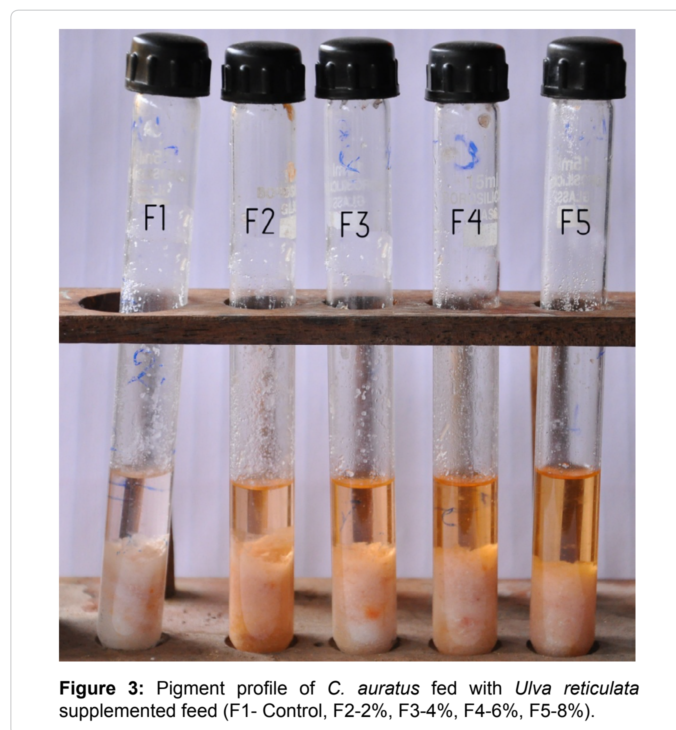


**Figure 3:** Pigment profile of *C. auratus* fed with *Ulva reticulata* supplemented feed (F1- Control, F2-2%, F3-4%, F4-6%, F5-8%).

| F1 | F2 | F3 | F4 | F5 | |
|---|---|---|---|---|---|
| 0.42 ± 0.070 | 0.51 ± 0.2 | 0.56 ± 0.11 | 0.57 ± 0.17 | 0.60 ± 0.07 | |
| **One way ANOVA for carotenoid content of Carassius auratus feed with different experimental diets** | | | | | |
| **Source of variation** | **SS** | **DF** | **MS** | **F** | **F Crit** |
| Between group | 97.51 | 4 | 24.38 | | |
| Within group | 298.6 | 10 | 29.86 | | |
| TOTAL | 396.11 | 14 | | | |

*F1-Control, F2-2%, F3-4%, F4-6%, F5-8%.

**Table 5:** Carotenoid content (µg/g wet weight) of *Carassius auratus* fed with different experimental diets.

| Pathogens | Solvent | |
|---|---|---|
| | **Methanol** | **Ethyl Acetate** |
| *Pseudomonas aeruginosa* | 10 mm | 7 mm |
| *Aeromonas hydrophila* | - | - |

**Table 6:** Antibacterial activity of seaweed extracts of *Ulva reticulata* against two bacterial fish pathogens.

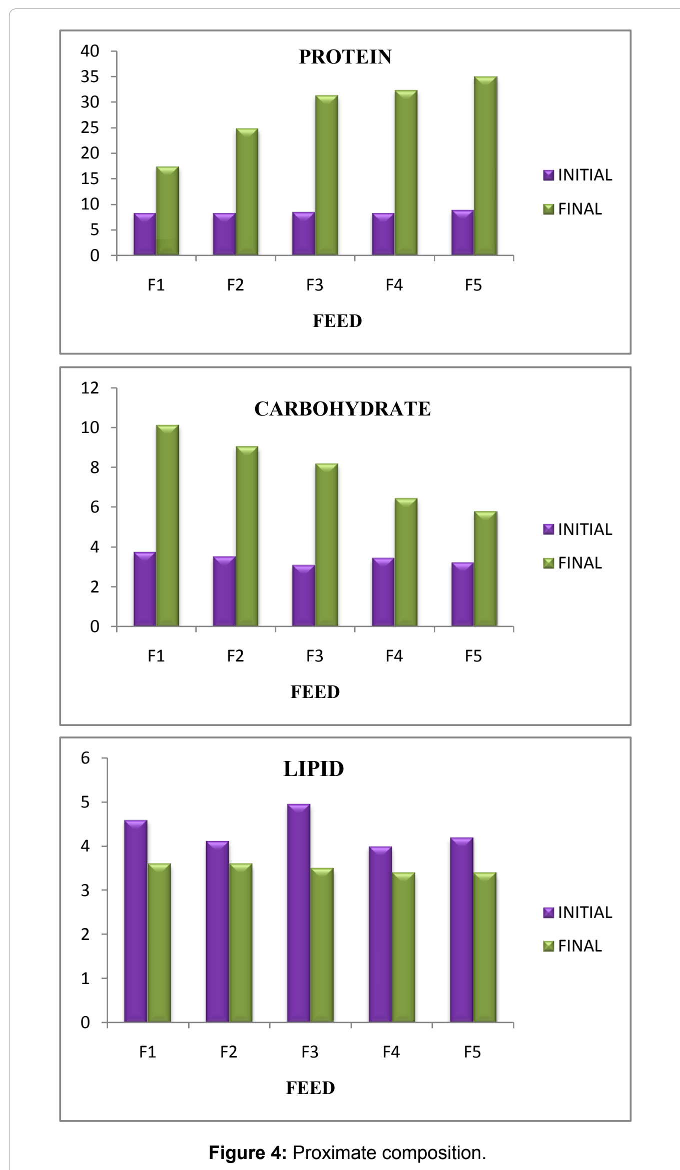


**Figure 4:** Proximate composition.

Growth performance in rainbow trout *Oncorhynchus mykiss* was reported to be reduced when fed on *U. lactuca* and *Enteromorpha linza* meal. Onder Yildirim, attributed the decline in growth performance of *Oncorhynchus mykiss* fed on *U. lactuca* and *Enteromorpha* diets when compared to group fed the non- algal diet to significantly higher fiber content [19]. Ibrahim, demonstrated increase in growth performance and higher body protein gain concentration for common carp *Cyprinus carpio* fed on dietary *Ulva rigida* meal supplementation in higher concentration (upto 15%) [47]. Bindhu and Shoba, reported very good response in terms of growth and feed utilization in *Ctenopharyngodon idella* fed with algal diet containing *Ulva fasciata, Spyridia insignis* and *Sargassam wightii* [48].

Ray and Das, successfully incorporated composted *Salvinia cuculata* until 20% dietary rate without compromising the growth performance in *Labeo rohita* [49]. Bairaji, concluded that feeding rohu *(Labeo rohita*) fingerlings with diet incorporating upto 30% fermented duckweed (*Lemna polyrhiza*) leaf gave better growth response, feed efficiency, higher protein and lipid gain compared to 10% raw duckweed inclusion level contrastingly catla *(Catla catla*) fingerlings fed with increasing amount of dietary *Spirogyra* sp meal [50], belonging to the class *Chloraphyceae* showed worse growth performance even at the lowest dietary inclusion level [51]. Studies of Chitra showed that fishmeal mixed with the marine algae powder *Sargassum wightii* gave better growth and weight increments of *Oreochromis mossambicus* [52].

Jafri and Farrog Anwar, stated that protein from plant sources showed higher digestibility than animal protein [53]. Supplementation of 5% *Ulva* meal for *Nile tilapia* was used in their study with no negative effect on growth performance, feed utilization and body composition. However; negative consequences resulted from using levels between 10% and 20% [54,55]. In contrast, Onder Yildirim, reported a decline in feed utilization and growth performance of rainbow trout fed diets with *Ulva lactuca* and *Enteromorpha linza* when compared with fish fed (10% level) a control diet [19]. A carnivorous fish like trout would accept rather animal origin ingredient than plant food stuffs. The growth retardation was attributed to the antinutritional characteristics of seaweeds.

In the present investigation, in gold fish being omnivorous the algal meal elicited a growth promoting effect. This may be due to their high water stability and lower disintegration, which are limiting factors in aquaculture as carp are slow feeders. Olin observed that the incorporation of the marine algae in formulated diets increases the water stability of pellets because of the nature of the phytochemical and stabilizers present in them [56]. *Ulva* fed fish displayed a marginal improvement in carcass protein. Our reports are in conformity with the finding of Sebshattin, Erugan who reported a similar observation in body composition of juvenile *Nile tilapia* (*Oreochromis niloticus*) fed on *Ulva* meal [57]. Reports of Guroy and Azaza also revealed an enhancement in carcass composition of *Nile tilapia* fed on *Ulva* meal [54,55]. Onder Yildirim reported a significant increase in the crude protein content of *Oncorhynchus mykiss* fed on *U.lactuca* and *Enteromorpho linza* [19].

Inclusion of algae meal had reduced lipid content of gold fish. Our reports are in conformity with Onder Yildirim who reported a similar reduction in lipid content of *Oncorhynchus mykiss* fed diet with *U.lactuca* and *Enteromorpho linza* [19]. The effect of dietary algal meal on carcass lipid can be contradictors, where as some reports suggest a decrease [56,57]. The effective substance provided by algae is not known but protein, fiber, mineral and trace elements need to be considered. The observed benefits may be explained by the high vitamin C content of *Ulva* meal [58,59] which affect lipid metabolism and may result in alternative of body composition and nutrient deposition in fish [60,61].

An observation of considerable interest during this study was increase in the carotenoid content of the test fish fed on *Ulva* supplemented diets. *Ulva* sps contain various pigments such as beta carotene, chlorophyll a, b and xanthophylls [62] which may possibly cause the enhancement of carotenoid pigments. *Ulva,* a natural pigment source may enhance the potential of seaweed inclusion in fish feed and may perhaps replace and reduce artificial colourants currently used by the industry [63]. Seaweed meal was found to be an effective colour enhancer at a cheaper price. Anna Soler-vila et al reported dark orange pigmentation in *Oncorhynchus mykiss* fed on red algae *Porphyra* compared to the control fish [64]. Ezhil found maximum carotenoid content in red sword tail *Xiphophorus helleri* fed on 15 gms of marigold pellet meal (6 g/100 g) [65].

A proportionate increase in carotenoid content in the fin, skin and

muscle with dietary supplemention of *Spirulina* in *Xiphophorus helleri* was reported by James [13]. Similarly, Relul Guroy, demonstrated an enhancement in yellow cichlid fed the diet containing *Spirulina* meal [66]. The dietary supplementation of algae was proved to enhance colour in fish. Enhancements of colour in fish fed algal diets are well documented [48]. The RBC counts were higher for the fish fed on 8% algal diets which was similar to the finding of Terry that striped tilapia fed with algae had a red blood cell count of 1.91-2.83×$10^6$ cell/ml [67]. Feeding *Spirulina plantensis* in processed form enhanced specific and non-specific immunity and resistance against *Edward ictaluis* infection in channel cat fish, *Ictaluras punctatus* [68].

James et al reported an increase in the monocytes, neutrophils and lymphocytes with increase in dietary level of *Spirulina* with 8% level fed group the maximum [13]. Similarly, fingerlings of *Clarias gariepinus* fed 5% *Spirulina* diet recorded higher values for red and white blood cell counts and the lysozymes activity [69]. Increased in WBC indicate that *Ulva* in the diet may benefit the immune system in *C. auratus* as well. *U. reticulata* contains carotenoids, which affect the health of fish, specifically improving health and increasing the ability to fight off infections through the reduction of stress level [70]. Antimicrobial activity depends on both algal species and the solvents used for their extraction. Similar to our finding, Periasamy Mansuya recorded the maximum activity (45 mm) from 200 mg aqueous extract of *Ulva reticulata* against *Salmonella typii* and minimum 9 mm by *Ulva lactuca* [71]. *Streptococcus pyogenes* at 50 mg level whereas, the methonalic extract showed the maximum activity (40 mm).

Seeenivasan, performed antibacterial activity studies *in vitro* with 3 extracts namely acetone, methanol and ethanol [72]. They observed that *Ulva fasciate* in selective media produced good results against *E.coli*. Cox et al screened the microbial activity of 6 species of edible seaweeds on food pathogenic bacteria such as *Listeria monocytogenus*, *Salmonella abony*, *Enterococcus faecalis* and *Pseudomonas aerugenosa* [31]. They found that dried methanol extracts of red and green seaweeds had significantly lower antimicrobial activity than brown seaweed.

Studies of Felix and Rajeev showed better inhibitory activity of *Graiularia verrucosa* against both *Vibrio alginolyticus* and *V. parahaemolyticus* [73]. Kolinjinathan and Stella reported zone of inhibition of *Ulva reticulata* and *U. lactuca* extract against gram positive and gram negative bacteria in the range of 7 mm to 16 mm [74]. the methanol extract showed highest mean zone of inhibition (15 ± 0.6 mm) against the gram positive cocci *Streptococcus pyogenes* followed by *Staphylococcus aureus* (13 ± 0.3 mm), *Streptococcus epidermis* (12 ± 0.6 mm), *Bacillus subtilis* (11 ± 0.6 mm) and *Bacillus cereus* (10 ± 0.55 mm). For gram negative bacteria, maximum zone of inhibition was recorded in methanol extract of *Ulva reticulata* against *Klebsiella pneumonia* (13+0.6 mm) followed by *Escherichea coli* (12+0.8 mm), *Enterobacter aerogenes* (10+0.03 mm), *Vibrio cholarae* (9+0.6 mm) and *Salmonella typhi* (9+0.3 mm). The zone of inhibition obtained from the hexane extracts of seaweed *Ulva reticulata* against bacterial pathogens was comparatively very less when compared to the other solvent extracts.

Alang, isolated eight compounds from *Ulva lactuca* for their antimicrobial activities and concluded that different extracts and fractions show moderate to significant microbial activity to the tested bacterial *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *E. coli* [75]. Highest zone of inhibition of 10mm in methanol extracts of green algae *Pithophora oedogonia* against gram negative bacteria, *Bacillus subtilis* and *Staphylococcus aureus* was observed by Anand Prakesh Singh and Chaudhary [76]. Radika, suggested that brown seaweed had high antibacterial activity against *Klebsiella* [77]. Antimicrobial activity of *U. lactuca* was correlated to its active compounds such as carbohydrates, steroids and glycosides [75].

Karthigai Devi, evaluated for antibacterial activity of commonly occurring green algae *Codium adherens*, *Ulva reticulata* and *Halimeda tuna* [78]. The ethanol extract was found to be more effective than other. Vallinayagam, screened the antibacterial activities of two important seaweeds namely *Ulva lactuca* and *Gracilaria edulis* were screened against human bacterial pathogens *Staphylococcus aureus, Vibrio cholerae, Shigella dysentriae, Shigella boydii, Salmonella paratyphi, Pseudomonas aeruginosa* and *Klebsiella pneumonia* [79]. The maximum activity was recorded from the extract of *Gracilaria edulis* against *Staphylococcus aureus* and minimum by *Ulva lactuca* against *Pseudomonas aeruginosa*.

## Conclusion

The present study of anti-bacterial activity of seaweeds *Ulva reticulata* extracts showed promising antimicrobial activity against fish pathogens, suggesting their potential for the development of antipathogenic agents in aquaculture. The enhancement in growth rate, colouration and hematological parameters observed in the test fish could be due to the disease resistance imparting antibacterial substances present in the *Ulva reticulata*.

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**References**

1. Dey VK (1996) Ornamental fishes and Handbook of Aqua farming The Marine Products Export Development Authority. Cochin.
2. Dibyendudutta A, Banerjee S (2012) Indigenous ornamental fishes of West Bengal. Recent research in science and technology 4: 12-21.
3. FAO (2004) Food and Agricultural Organization, Fisheries Information, Rome, Italy.
4. Threatened freshwater fishes of India (2010) National Bureau of Fish Genetic Resources. ICAR. Lucknow, India.
5. Paripatanamont T, Tangtrongpairoj J, Sailasuta A, Chansue N (1999) Effect of astaxanthin on the pigmentation of goldfish Carassiusauratus. J World AquacultSoc 30: 454-460.
6. Gouveia L, Rema P (2005) Effect of microalgal biomass concentration and temperature on ornamental goldfish (Carassiusauratus) skin pigmentation. Aquacult Nutr 11: 19-23.
7. Saxena A (1994) Health, colouration of fish. International Symposium on Aquatic Animal Health: Program and Abstracts. University of California, School of Veterinary Medicine, Davis, CA, USA.
8. Goodwin TW (1984) The Biochemistry of Carotenoids Volume II. Chapman and Hall, London, UK.
9. Hata M, Hata M (1973) Studies on astaxanthin formation are some freshwater fishes. Tohoku Journal of Agricultural Research 24: 192-196.
10. Coliheuque N (2010) Genetics of salmonid skin pigmentation: clues and prospects for improving the external appearance of farmed salmonids. Rev Fish Bio Fisheries 20: 71-86.
11. Xu X, Jin Z, Wang H, Chen X, Wang C, et al., (2006) Effect of astaxanthin from Xanthophyllomyces dendrorhous on the pigmentation of goldfish, Carassius auratus. Journal of World Aquacult Soc 37: 282-288.
12. Bahmani M, Kazemi R, DonskayaP (2001) A comparative study of some haematological features in young reared sturgeons (Acipenserpersicus and Husohuso). Fish PhysiolBiochem 24: 135-140.
13. James R, Sampath K, Thangarathinam R, Vasudevan I (2006) Effect of

dietary Spirulina level on growth, fertility, coloration and leucocyte count in red swordtail, Xiphophorushelleri Isr J Aquacult Bamidgeh 58: 97-104.

14. Promya J, Chanagun C (2011) The effects of Spirulinaplatensis and Cladophora algae on the growth performance, meat quality and immunity stimulating capacity of the african sharptooth catfish (Clariasgariepinus). International Journal of Agriculture and Biology: 1814-9596.
15. Mustafa MG, Nakagawa H (1995) A review: dietary benefits of algae as an additive in fish feed. Isr Journal Aquacult Bamidgeh 47:155-162.
16. Hashim R, MustalaSaat MA (1992) The utilization of seaweed meals as binding agents in pelleted feeds for snakehead (Channastriatus) fry and their effects on growth. Aquaculture 108: 299-308.
17. Hardy RW, Tacon AGJ (2002) Fish meal: historical uses, production trends and future outlook for sustainable supplies. In Stickney RR, McveyJp (ed) Responsible marine aquaculture. CABI International 311-325.
18. Nakagawa H, Kasahara S, Sugiyama T (1987) Effect of Ulva meal supplementation on lipid metabolism of black sea bream Acanthopagrusschlegeli (Bleeker). Aquaculture 62: 109-121.
19. Yildirim O, Ergun S, Yamam S, Tuker A (2009) Effect of two seaweeds (Ulvalactuca and EnteromorphaLinza) as a feed additive in diets on growth performance, feed utilization and body composition of Rainbow trout (Oncorhynchusmykiss). Kafkas Univ Vet FakDerg 15: 455-460.
20. Hassim R, Hassan HN (1995) The use of various level of Ulvasp meal as binders for practical and their effect on growth of snakehead (Channastriatus) fry. Journal Bioscience 6: 123- 131.
21. Mustafa MG, Wakamatsu S, Takeda T, Umino T, Nakagawa H (1995) Effects of algae meal as a feed additive on growth performance, feed efficiency, and body composition in red sea bream. Fish Sci 61: 25-28.
22. Wassef EA, El- Masry MH, Mikhail FR (2001) Growth enhancement and muscle structure of striped mullet (Mugilcephalus L.) fingerlings by feeding algal meal-based diets. Aquac Res 32: 315-322.
23. Nakagawa H, Nematipour RG, Yamamoto M (1993) Optimum level of Ulva diet supplement to minimize weight loss during wintering in black sea bream (Acanthopagrusschlegeli). Asian Fish Sci 6: 139-148.
24. Michael TM, John M, Jack P (2005) Brock Microbiology of Microorganisms. 11th Edition New Jersey, USA.
25. Tuney I, Cadirci BH, Unal D, Sukatar A (2006) Antimicrobial activities of the extracts of marine algae from the coast of Urla (Izmir, Turkey). Turkish Journal of Biology 30: 171-175.
26. Serkedjieva J (2004) Antiviral activity of the red marine algae Ceramiumrubrum. Phytotherapy Research 18: 480-483.
27. Garg HS, Sharma T, Bhakuni DS, Pramanik BN, Bose AK (1992) An antiviral sphingosine derivative from green alga Ulvafasiata. Tetrahedron Letters 33: 1641-1644.
28. Tang HF, Yi YH, Yao XS, Xu QZ, Zhang SY, et al., (2002) Bioactive steroids from the brown algae Sargassumcarpophyllum. Journal of Asian Natural Product Research 4: 95-105.
29. Thangam TS and Kathiresan K (1991) Mosquito larvicidal activity of marine plant extracts with synthetic insecticides. Bot Mar 34: 537-539.
30. Tukmechi A, Ownagh A, Mohebbat A (2010) In vitro antibacterial activities of ethanol extract of Iranian propolis (EEIP) against fish pathogenic bacteria (Aeromonashydrophila, Yersinia ruckeri& Streptococcus iniae). Brazilian Journal of Microbiology 41: 1086-1092.
31. Cox S, Abu-Ghannam N, Gupta S (2010) An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. International Food Research Journal 17: 205-220.
32. Hardy R (1980) Fish feed formulation. In: Fish Feed Technology ADCP/REP/80/11, FAO, UN, Rome.
33. Lowry OH, Rosebrough NJ, Farr AL and RJ Randall (1951) Protein measurement with Folin phenol reagent. Journal Biol Chem 193: 265-275.
34. Bragdon JH (1951) Colorimetric determination of blood lipids. Journal Biol Chem 190: 513-517.
35. Lovell RT (1981) Laboratory manual for fish feed analysis and fish nutrition studies. Dept Fisheries Allied Aquacult, Int Center for Aquacult, Auburn University, Alabama, USA.
36. Olson JA (1979) A simple dual assay for vitamin A and carotenoids in human and liver. Nutrition Report Journal 19: 807-813.
37. Raymont JE, Austine GA, Lingfold E (1964) Biochemical studies on marine zooplankton. The biochemical composition of Neonysisinterger. Journal Cons Internationals Explor Mer 28: 354-563.
38. Seifter SS, Dayton BN, Mantwyler E (1950) The estimation of glycogen with anthrone reagent. Arch bio chem Biophysics 25: 190-239.
39. Yokoyama HO (1947) Studies on the origin, development and seasonal variations in the blood cells of the perch, Percaflavescens. PhD Thesis, University of Wisconsin, Madison, Wisconsin 144.
40. Christensen GM, Faindt JT, Poeschi BA (1978) Cells and certain physiological properties of brook trout (Salvelinusfontinalis) blood. Journal of Fish Biology 12: 51-60.
41. Bauer AW, Kirby WMM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. American Journal Clinical Pathology 45: 493-496.
42. Snedecore GW, Cochran WG (1967) Statistical methods 6th edi. Lowa State Univ Press Lowa.
43. Zar JH (1984) Biostatistical Analysis, 2nd ed. Prentice-Hall IntInc Englewood Cliffs, New Jersey 224-260.
44. Mensi F, Jamel K, Amor EA (2005) Potential use of seaweeds in Nile tilapia (Oreochromisniloticus) diets. Meditarranean fish nutrition 151- 154.
45. Betoikut Guroy K, Cirik S, Guroy D, Sanver F, Tekinay AA (2007) Effects of Ulvarigida or Cystoseirabarbata meals as a feed additive on growth performance, feed utilization, and body composition in Nile tilapia, Oreochromisniloticus. Turk J Vet Anim Sci 31:91-97.
46. Kissil GW, Lupatsch I, Higgs DA, Hardy RW (2000) Gilt head sea bream Sparusaurata. In: Stickney, RR (ed) Encyclopedia of aquaculture, Johnwiley and sons, Newyork 409-416.
47. Ibrahim Diler A, Adem A, Tekinay A, Deryaguroy, Soyuturk M et al, (2007) Effect of Ulvarigida on the growth, feed intake and body composition of common carp L. Journal of Biological Sciences 7: 305-308.
48. Bindu MS, Sobha V (2005) Impact of marine algal diets on the feed utilization and nutrient digestibility of grass carp. Ctenopharyngodonidella ecology and noospherology T 16 No 1-2.
49. Ray AK, Das I (1992) Utilization of diets containing composted aquatic weed (Salviniacuclata) by the Indian major carp, rohu (Labeorohita Ham) fingerlings. Bioresour technol 40: 67-72.
50. Bairaji A, SarkasGhosh K, Sen SK, Ray AK (2002) Duckweed (Lemnapolyrhiza) leaf meal as a source of feed stuff in formulated diets for rohu (Labeorohita Ham) fingerlings after fermentation with a fish intestinal bacterium. Bioresour technol 85: 17-24.
51. Harish Kumar M, Gajaria SC, Radha KS (2004) Growth and development of catla (Catlacatla) fed with different level of diet containing Spirogyra sp. Bioresour technol 95: 73-76.
52. Chitra G (1996) Biochemical studies of algae along the coast of Kerala with special reference to industrial application. PhD Thesis, University of Kerala 183.
53. Jafri AK, M Farooq A (1995) Protein digestibility of some low-cost feed stuffs in fingerling Indian major carps. Asian journal of Fisheries Science 8: 47-53.
54. Guroy BK, Cirik S, Guroy D, Sanver F, Tekinay AA (2007) Effects of Ulvarigida or Cystoseirabarbata meals as a feed additive on growth performance, feed utilization, and body composition in Nile tilapia, Oreochromisniloticus. Turk J Vet Anim Sci 31: 91-97.
55. Azaza MS, Mensi F, Ksouri J, Dhraief MN, Brini B, et al, (2008) Growth of Nile tilapia (Oreochromisniloticus L) fed with diets containing graded levels of green algae Ulva meal (Ulvarigida) reared in geothermal waters of southern Tunisia. Journal of Applied Ichthyology 24: 202-207.
56. Olin KL, Golub MS, Gershwin MC, Hendrickx AG, Lonnerdal B et al, (1995) Extracellular superoxide dimustase activity is affected by dietary Zinc intake in non-human primate and vodent models. American Journal of Clinical Nutrition 61: 1263-1267.

57. Ergun S, Soyuturk M, Guroy B, Guroy D, Merrifield D (2008) Influence of Ulva meal on growth, feed utilization and Body composition of juvenile Nile tilapia (Oreochromisniloticus) at two levels of dietary lipid. Aquaculture International 17: 355-361.

58. Ortiz J, Romero N, Robert P (2006) Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds Ulvalactuca and Durvillaeaantarctica, Food Chemistry 99: 98-104

59. Garcıa Casal MN, Pereira AC, Leets I, Ramırez J, Quiroga MF (2007) High iron content and bioavailability in humans from four species of marine algae. Journal of Nutrition 137: 2691-2695.

60. Nakagawa H, Mustafa MG, Takii K, Umino T, Kumai H (2000) Effect of dietary catechin and Spirulina on vitamin C metabolism in red sea bream. Fish Sci 66: 321-326.

61. Ji H, Om A, Yoshimatsu T, Hayashi M, Umino T, et al, (2003) Effect of dietary vitamins C and E fortification on lipid metabolism in red sea bream Pagrus major and black sea bream Acanthopagrusschlegeli. Fish Sci 69: 1001-1009.

62. Bindu MS, Sobha V (2006) An Appraisal of Seaweed resource of Kerala coast. Algae in Biotechnology and Environment, International Conference on Applied Phycology, University of Del.

63. Nickell DC and NR Bromage (1998) The effect of timing duration of feeding astaxanthin on the development and variation of fillet colour and efficiency of pigmentation in rainbow trout (Oncorhynchusmykiss). Aquaculture 169: 233-246.

64. Soler- Vila A, Coughlan S, Michael D, Guiry, Kraan S (2009) The red alga Porphyradioica as a fish-feed ingredient for rainbow trout (Oncorhynchusmykiss): effect on growth, feed efficiency and carcass composition. J Appl Phycol 21: 617-624.

65. Ezhil J, Jeyanthi C, Narayanan M (2008) Marigold as a carotenoid source on pigmentation and growth of Red Sword Tail, Xiphophorushellari. Turkish Journal of fisheries and Aquatic sciences 8: 99-102.

66. Relul Guroy D, Sahin I, Guroy B, Altin A, Merrifield DL (2012) Effect of dietary protein level on growth performance and nitrogen excretion of yellow tail cichlid Pseudotropheusacei.

67. Terry CH, Cardinale JL, Smith SA (2000) Haematology and plasma chemistry reference intervals for cultured tilapia (Oreochromis hybrid). Vet Clin Pathol 29: 7-12.

68. Duncan PL, Kiesius PH (1996) Effects of feeding Spirulina on specific and non-specific immune responses of channel catfish. J Aquatic Animal Health 8: 308-313.

69. Vonshak A (1997) Spirulinaplatensis (Arthospira): Physiology, Cell Biology and Biotechnology. Taylor and Francis, London.

70. Nakono T, Yamaguchi T, Sato M, Iwama GK (2003) Biological Effects of Carotenoids in Fish. International Seminar, Effective Utilization of Marine Food Resource, Songkhla, Thailand.

71. Mansuya P, Aruna P, Sridhar S, Jebamalai SK, Babu S (2010) Antibacterial activity and qualitative phyto chemical analysis of selected seaweeds from Gulf of Mannar region. J Exp Sciences 1: 23-26.

72. Seenivasan R, Indu H, Archana G, Geetha S (2010) The Antibacterial activity of some marine algae from south east coast of India. American-Eurasian J Agric & Environ Sci 9: 480-489.

73. Felix S, Rajeev A (2004) Immune enhancement assessment of dietary incorporated marine algae Sargassumwightii (Phaeophyceae/Punctariales) in tiger shrimp Penaeusmonodon (Crustacea/Penaeidae) through prophenoloxidase (proPO) system. Indian J Mar Sci 33: 361-364.

74. Kolinjinathan K, Stella D (2011) Comparative studies on antimicrobial activity of Ulvareticulata and Ulva lactuca against Human Pathogens. Internatinal Journal of Pharmaceutical and Biological Archives 2: 1738-1744.

75. Alang G, Kaur R, Singh A, Budlakoti P, Singla P (2009) Antimicrobial activity of Ulvalactuca extracts and its fractions. Pharmacollogyonline 3: 107-117.

76. Singh AP, Chaudhary BR (2010) Analysis and In vitro Antibacterial screening of Pethophoraoedogonia (Mont) Wittrock- A Preliminary Phycochemical Freshwater Green Alga Forming Mats in the Water Bodies. J Algal Biomass Utln 1: 33-41.

77. Radhika D, Veerabahu C, Priya R (2012) Antibacterial activity of some selected seaweeds from the Gulf of Mannar. Asian J Pharm Clin Res 5: 89-90.

78. Karthikai Devi G, Thirumaran G, Manivannan K and P Anantharaman (2009) Element composition of certain seaweeds from Gulf of Mannar marine biosphere reserve: Southeast coast of India. World Journal of Dairy and Food Sciences 4: 46-55.

79. Vallinayagam K, Arumugam R, Kannan RRR, Thirumaran G, Anantharaman P (2009) Antibacterial activity of some selected seaweeds from Pudumadam Coastal Regions. Global J Pharmacol 3: 50-52.