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Studies on effect of marine macro algae *Enteromorpha intestinalis* as skin color enhancer and as an alternative for fish meal in feed supplemented to ornamental fishes

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Abstract

Dried powder of marine algae *Enteromorpha intestinalis* has been found as a potent skin color enhancer and growth promoter in freshwater ornamental fishes. Juveniles of Guppies and Rosy barb 30 fishes (10 fishes in each glass bowl) were fed with macroalgae at 1%, 2% and 3% concentration level through fishmeal based supplementary diet for a period of 30 days. The study was an attempt to observe the responses of *Enteromorpha* diet towards skin color development and muscle growth pattern of the culture species as well as the probable economic returns obtained from the experiment in order to commercialize the particular product in local aqua feed market. The desired level of macro algae administration produced animals with significantly ($T < 0.05$) higher body weight. In general, the carotenoid-supplemented diets significantly increased the values of redness (a^*), yellowness (b^*), and chroma (C^*), and decreased the values of lightness (L^*) and hue ($H^{p_{ab}}$) on the tail, body, and head areas ($p < 0.05$). Beta carotene present in algae enhanced color in fishes (0.06%). An average weight gain of 25.56% and compared to control diet fed fishes (length-5.4cms) length of 7.8cms increase was obtained with inclusion of *Enteromorpha intestinalis* in the diet which may be a direct consequence of hypertrophic muscle growth pattern observed in the species. The present findings thus indicate that macro algae may be used as a cost-effective natural pigment enhancer (alternative carotenoid source) as well as growth promoter in fish feeds for higher profitability, good color enhancement (carotenoid content of muscle was $0.179 \pm 0.1\%$ whereas for control group of fishes carotenoid was $0.146 \pm 0.1\%$), better growth (initial body weight of $0.54 \pm 0.14g$ and final body weight of $6.34 \pm 0.27g$ when compared to control group fishes initial body weight of $0.47 \pm 0.12g$ and final weight $4.23 \pm 0.12g$)

Keywords: *Enteromorpha intestinalis*, carotenoid, aqua-feed, Body weight, skin color enhancement, nutritive analysis

Introduction

Ornamental fishes are reared for setting up aquarium which is in fact a hobby for many people all around the world. Aquarium fishes give a feel of bliss and relaxation and provide feast to the watching eyes. Aquarium is not a new phenomenon, it exists from 1970's onwards right from British era. (Palaparthi Anil Kumar et al., 2017) [21]. India has a diversity of ornamental fishes which are reared for aquarium purpose. (Ghosh. A et al., 2003) [22]. Ornamental fish culture is eco-friendly and is of low investment and good profit yield is obtained as there is always a growing demand for these aquarium fishes everywhere. (Anusha. P et al., 2014) [23]. Color plays a major role in aquarium fishes and the price of fishes depends on the color of the fish as well as the size of the fish. (Harpaz 2007) [24].

Nutritionally balanced fish feeds generally contain fishmeal, soy products meal, Tapioca, Turmeric, Garlic, cornstarch, vitamin, mineral, and algae. Investigations for cheaper alternative feedstuffs as protein and energy source for fish diets have become most important in order to produce low-cost pelleted feeds available for the small-scale farmers both in developed and developing countries. Continuously increasing demand for fish feed has paved way for consideration of every possible natural resource as a potential ingredient in fish feed. Algal meals are an alternative plant feedstuffs that are increasingly being used in aqua feeds because of their nutritional quality, lower cost and availability (Mustafa and Nakagawa, 1995) [25]. *Ulva* sp. belongs to the class ulvophyceae.

The specific species of *Ulva intestinalis* is grown in high mass. In a study conducted by Siddiq *et al.*, 2015 shows *Ulva* sp. to be rich in nutritive value; hence, could serve as an alternative fish feed ingredient. The main aim of this study is to replace fish meal with algal diet and to enhance pigmentation in the ornamental fishes.

Materials and methods

Collection and processing of marine algae

Sample Collection

Fresh green seaweed sample of *Enteromorpha intestinalis* were collected from rocky shores in Kovalam, Chengalpattu district in the month of October to November 2019 during low tide. The algal samples were handpicked during low tide in rocky areas and washed in sea water to remove all unwanted impurities, epiphytes, animal castings and adhering sand particles etc. The algae were placed separately in new polythene bags and kept in an ice box and transported to the laboratory. The collected seaweeds were washed with fresh water to remove associated debris and epiphytes. The cleaned materials are air dried under shade at 30°C. The dried samples were finely powdered and stored at 20°C.

The seaweed powder was mixed with fishmeal at a level of 3% within the formulated diet to meet the nutritional requirements of fish. Simultaneously a control diet was also

formulated with 1% inclusion of seaweed powder. A 30 days growth trial was conducted, fish juveniles of mean initial weight of 2.3g were obtained from a local hatchery and acclimated to the conditions prior to initiation of the experiment. Stocking at a density of 2 individuals/m² was done in the experimental ponds. Dietary treatments were randomly assigned to the ponds and feeding on the respective diets was initiated.

Sampling

Fish samples were collected at every 10 days interval. Body weight was measured with the help of field electronic balance prior to sacrificing the animals. The feed conversion ratio (FCR) was calculated as,

FCR = estimated individual consumption / average individual increase in weight.

The survival rate was calculated as: (final number / initial number) × 100, for each experimental bowl.

Statistics

To explore the relationships between body weight and muscle fibre expansion, equations were computed. All the collected data were subjected to Analysis of Variance (ANOVA) and the calculations were performed with Web Agri Stat Package (WASP) statistical software for Windows.

Table 1: Proximate Analysis: Final carcass (g Kg⁻¹)

Components	Control diet fed fishes	1% algal diet fed fishes	2% algal diet Fed fishes	3% algal diet fed fishes.
Dry matter	328.2	325.2	327.8	330.5
Crude protein	236.4	225.3	230.9	248.7
Crude fat	87.4	72.6	71.4	70.1
Crude fiber	52.1	54.7	56.8	59.4
Ash	117.2	123.4	128.5	132.7
Energy	2574.2	2641.5	2674.6	2692.7
Total	500.0	500.0	500.0	500.0

Diet preparation

Three practical diets were formulated to provide 37 to 40 % crude protein and 9 - 10 % crude fat containing varying amount of dried raw *E. intestinalis*. The dried raw seaweed replaced soybean meal by weight at 3% (Control diet), 6% (Diet 2) and 9 % (Diet 3). These weight replacements were equivalent to 0 %, 3.1 % and 6.2 % of soybean protein replacements, respectively.

The diets were formulated following that of Santiago *et al.*,

1982. The ingredients were ground and sieved at 400µm prior to mixing; all dried ingredients were thoroughly mixed and liquid ingredients and vitamin/ mineral premix added last. Gelatinized Corn starch was added as a final step before pelletizing. The moistened mixture was pelleted (2 mm) in a meat grinder and oven-dried (60° C) long enough to reduce moisture to less than 10 %. Diets were crumbled into different pellet sizes (0.5 to 3.0 mm), sealed in plastic bags, and stored at -20° C until use.

Table 2: Diet formulation

Ingredients	Control diet % (0%)	Diet 1(3%)	Diet 2(6%)	Diet 3(9%)
Fish meal	10%	-	10%	-
Tapioca	5%	5%	5%	5%
Garlic	5%	5%	5%	5%
Turmeric	5%	5%	5%	5%
Vegetable oil	2%	2%	2%	2%
Vitamin & mineral mix	2%	2%	2%	2%
Soyabean meal	1%	1%	-	-

Experimental fishes and set up.

50 fishes of Rosy barb (*P. conchonius*), Zebra danio (*D. rerio*) fry were procured from an aquarium in Chennai and acclimatized in the hatchery laboratory in a fibreglass bowl with continuous aeration for 10 days. During acclimatization, the fishes were fed with the control diet. Fish fry were randomly stocked in glass bowls (10 fry Per tank). The experimental diets were fed to fry (average body weight

ABW- of 0.03 ±0.00 g) at a rate of 4 times daily. Every 10 days, fish were bulk-weighed during the entire feeding trial and feed amount was adjusted continually. The whole feeding trial lasted for 30 days.

About 70 % of the water volume in the system was replaced and filters cleaned every 2 days. Uneaten feeds and faeces were siphoned off every morning before the first feeding. Chlorinated tap water (100 ppm NaCl) was subjected to

strong aeration 3 days before use. Water temperature and pH were measured twice a day, while dissolved oxygen (D.O. 5Mg/dL) was measured twice a week, and nitrite and total ammonia weekly using commercially available kits (AQUA-NITETM and AQUA-AMTM, respectively).

Growth performance parameters

Growth and feed utilization efficiency were calculated using the following formulas:

$$\text{Weight gain, WG (g)} = W2 - W1$$

Where

W2 = Final weight (g)

W1 = Initial weight (g)

$$\text{Specific Growth Rate (SGR, \% / day)} = [(\ln W2 - \ln W1) / (T2 - T1)] * 100$$

Where

W2 = Final weight (g)

W1 = Initial weight (g)

T2 = Final time (in days)

T1 = Initial time (in days)

$$\text{FCR} = \text{Feed intake (g)} / \text{Weight gain (g)}$$

$$\text{Nutrient Retention (\%)} = [(\% \text{ final carcass nutrient} \times \text{final$$

$$\text{ABW (g)} - (\% \text{ initial carcass nutrient} \times \text{initial ABW (g)}) \times 100 / \text{total nutrient intake (g)}$$

$$\text{Survival (\%)} = (\text{Survived fish} / \text{Initial fish stocked}) \times 100$$

Spectrophotometric Analysis

The method used for pigment extraction from the red sword tail tissue was as described by Olson, 1979. One gram of entire fish body tissue (without head and alimentary canal) was taken in 10 ml screw capped clear glass vials and 2.5 g of anhydrous sodium sulphate was added. The sample was gently mashed with a glass rod against the side of the vial. 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 which maximum absorption, was recorded was used for the calculation.

$$\text{Total carotenoid} = \frac{\text{Absorption at wave length} \times \text{Dilution factor}}{0.25 \times \text{sample weight in g}}$$

Where, Dilution factor = 10

Extinction co-efficient = 0.25

Weight

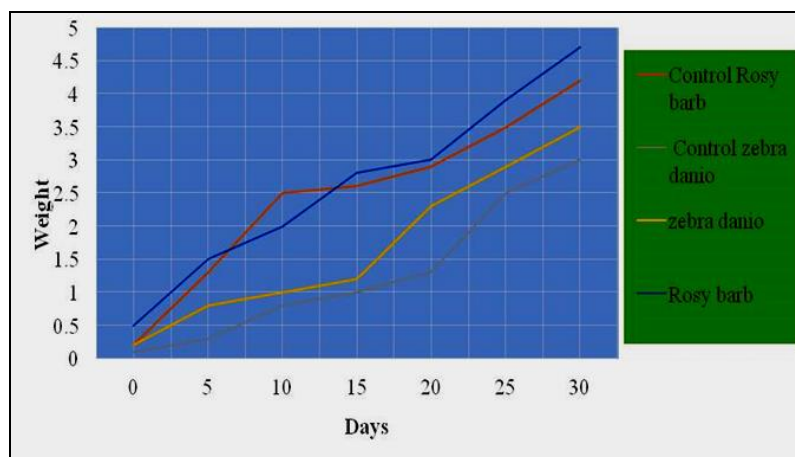


Fig 1: Graph representing comparison of weight in grams among the fishes

Length

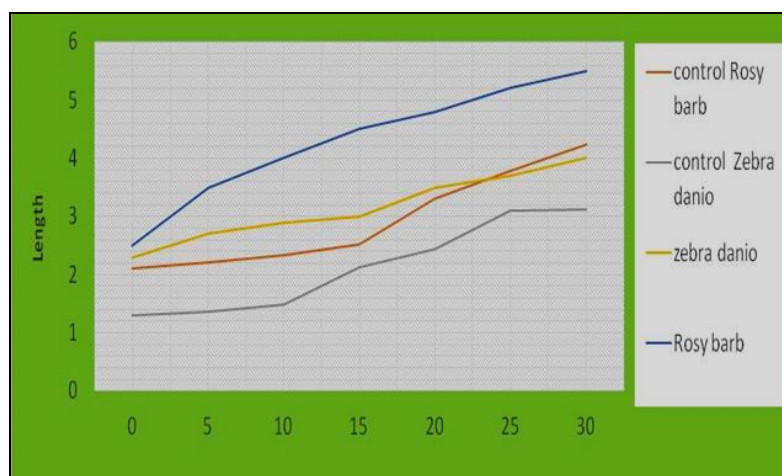


Fig 2: Graph representing comparison of length of body of fishes in centimeters

Results

Water quality

The following ranges of values for the water quality parameters were recorded: temperature $27.3 \pm 0.1^\circ\text{C}$; pH 9.0 ± 0.01 ; D.O. 10.1 ± 0.3 ppm; ammonia 0.02 ppm and nitrite 0.01 ppm. All measured parameters were within the optimal range known for the fishes.

Table 3: Determines the water quality parameters

S. No.	Parameters	Observation
1	pH	7.5
2	Alkalinity	440mg/mL
3	Hardness	200mg/mL
4	Chloride	200 mg/mL
5	TDS	100.8 mg/mL
6	Fluoride	1.5 mg/mL
7	Iron	Absence
8	Ammonium	0.5 mg/mL
9	Phosphate	Absence
10	Residual chloride	4 mg/mL

Growth performance and nutrient utilization

WG, SGR, and feed intake of fish fed with the diet containing 15 % dried *E. intestinalis* powder exhibited no significant difference with those fed with the control diet; fish fed diets with 30 % replacement by seaweed was significantly lower ($P \leq 0.05$) than the control and 15 % seaweed- replaced diets. Growth performance, feed and nutrient utilization of Rosy barb and Zebra Danio fed with the control and soybean replacement with raw dried *E. intestinalis* diets for 30 days. FCR and survival did not differ significantly among dietary treatments ($P \geq 0.05$). Fishes fed with the control diet exhibited higher protein and lipid retention than did fish fed diets with soybean meal replaced by *E. intestinalis*. However, those fish fed diets containing seaweed exhibited numerically higher body crude protein than did fish fed with the control diet. Protein retention of fish fed with the control and 15 % seaweed- replaced diet were not significantly different from each other ($P \geq 0.05$) while the 30 % seaweed- replaced diet was significantly lower. In contrast, lipid retention in fish fed with seaweed replaced diets were significantly lower ($P \leq 0.05$) than in those fed with the control diet.

Table 4: Chemical composition of raw dried *E. intestinalis*

S. No	Biochemical parameters	Composition (gKg- 1)
1	Dry matter	427.3
2	Crude protein	1.21
3	Crude lipid	46.0
4	Ash	230.5
5	Total	500

Discussion

From the study it has been discussed that the seaweed enteromorpha intestinalis is an effective color enhancer as well as growth promoter in aquatic fishes and could be used as an alternative feed supplement in the daily diet of aquarium fishes.

Conclusion

The overall experiment proved that there is good color enhancement in reared fishes Rosy barb and Zebra danio, thus *Enteromorpha intestinalis* could be suggested as the alternative feed and replacement for other protein feedstuffs as well as natural pigment enhancer.

Conflict of interest: The authors have no conflict of interest.

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