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Seaweed supplemented diet induced skin colour changes in freshwater ornamental fish *Puntius tetrazona*

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Abstract

Carotenoids are lipid soluble compounds. Therefore, the amount and type of lipid with which they are consumed may influence their absorption. In this study analyses the effect of seaweed supplemented diet and its skin colour changes in the skin of tiger barb and study the quantitative and qualitative analysis of skin using spectrophotometer and TLC. The present study was undertaken to study the effect of seaweed supplemented diet for different feeding duration (days) fed in tiger barb *Puntius tetrazona* to assess the enhancement of body colour. The total carotenoid content of the control fish skin sample was 2.917 µg/g tissue and which increased during experimentation from 4.0926 µg/g tissue and 5.1552 µg/g tissues. The trend was noticed in the skin of tiger barb fed with seaweed supplementation diet.

Keywords: *Puntius tetrazona*, carotenoids, supplemented diet, skin tissue

Introduction

The word seaweed gives the wrong impression that it is a useless plant on the contrary, seaweeds are highly useful plants. Seaweeds are simple plants growing in the shallow waters of the seas using solar energy to produce carbohydrates from Co_2, water and dissolved nutrients. In India, at present, the seaweeds collected from wild are used as raw materials for the production of agar, alginates and liquid seaweed fertilizer. The unexploited species of Gracilaria, Hypnea, Acantophora, Laurencia, Hormophysa, Ulva, Enteromorpha and Caulerpa may be harvested from their natural beds at different parts of Indian coast and used for the production of agar carrageenan, alginates and edible products.

On the other hand, information on the effect of different types of dietary lipid on carotenoid utilization is relatively spare. The general nature of the carotenoid pigments in many classes of algae is now reasonable well established (Goodwin, 1958a, 1959a,b and 1984) [9, 10]. However, little is known of the carotenoids in the class chrysophyceae, the only report available being that Heilbron (1942) [12] in which he reported β-carotene, lutein (3,3-dihydroxy-α-carotene) and fucoxanthin in a mixed culture of Apistonema carteri, Thallochrysis litoralis and Gloeschrysis maritime.

Seaweeds are now a days widely used in industry, but it is only recently that they have been recognized as potential sources of antimicrobial and antifungal substances (Pesando and Caram, 1984) [16]. Seaweed constitutes one of the commercially important marine living and renewable resources. They contain more than 60 trace elements, minerals, protein, iodine, bromine, vitamins and substances of stimulatory and antibiotic nature. Agar-agar, carragreenan and algin are the phycocolloids extracted from red and brown algae. These chemical constituents play a key role in metabolism of algae. Although the composition of organic constituents in marine algae has been studied for a few decades in the past, there are only a few instances of detailed analysis of mannitol and total carbohydrates in all the three groups (Green, Brown and Red) of algae (Black, 1954; Percival, 1968) [3].

The pigmentation level of an aquatic animal may be an important factor affecting is market value and may also directly indicate the healthiness and quality of an organism (Chien and Jeng, 1992) [6]. Esterified astaxanthin is also found in the skin of salmonids and can account for upto 20% of total carotenoid in the fish (Schiedt *etal.*, 1988 a, b) [19]. Thus many non-phototrophic bacteria and fungi relay on carotenoids for protection when growing in high and air (Britton *et al*., 1995) [4]. To analyse the effect of seaweed supplemented diet and its carotenoid changes in the skin of tiger barb and study the quantitative and qualitative analysis of skin using spectrophotometer and TLC. As fish cannot synthesize these pigments, they rely on dietary supply of carotenoids to achieve their natural skin pigmentation, one of the most important quality criteria informing the market value of ornamental high value species such as tiger barb (*Puntius tetrazona*) and goldfish. Additional, isoxanthopterin, neopterin, pterin, sepiapterin and xanthopterin are the most abundant pigments found in fishes (Leclercq *et al*., 2010) [13]. However, these are colorless or pale yellow pigments, so the golden color might be due to carotenoids rather than to pteridine groups.

Materials and Methods

In the present study seaweed induced carotenoid changes was analysed in tiger barb (*Puntius tetrazona*). For this two different experiments were performed at different feeding durations such as 0 and 15 days.In the experiment well acclimatezed fishes were fed with seaweed supplemented diets. The fishes were withdrawn after the experimental period and the tissue skin was dissected out under low temperature.

The collected tissues were used for further analysis.

Sample collection
In both control and experimental fishes samples were collected for further analysis. The skin samplewas collected and placed in a clean vial, marked and temporarily stored at low temperature (-20^0C) until used for further analysis.

Estimation of Protein (Lowry *et al.*, 1951) [14]
A know amount of the skin sample were taken and ground it well with 80% ethanol. Then it was centrifuged with 15 min. at 5000 rpm. The precipitate was then taken and dissolved in 1 N NaOH and made upto 5 ml. From this, 0.5 ml was thaken then 5ml of the solution C was added and kept it for 10 min. Finally 0.5 ml of folin phenol reagent was added and the intensity of colour developed was read at 640 nm in a Spectrophotometer.

Estimation of carbohydrate (Carrol *et al.*, 1951) [5]
The carbohydrate content in the control and experimental tissue samples of C. carpio was estimated by the Anthrone method described by Seifter *et al*. (1950). To an aliquot of tissue homogenate, 4 ml of anthrone reagent (0.2% in sulphuric acid) was added and was incubated in a boiling water bath for 15 minutes. Tubes were then cooled to room temperature at dark condition. Then the Optical Density was measured at 750 nm in a Spectrophotometer. Here glucose (100 mg/100 ml distilled water) was used as the standard.

Estimation of lipid (Folch *et al.*, 1957) [7]
Fifty milligram experimental tissue sample was taken and homogenized well with 4ml of chloroform methanol mixture. After mixing well, 0.2 ml of o.9% sodium chloride was added. Kept the mixture undisturbed overnight. The lower layer of lipid was collected carefully and dried in a vacuum dessicator. The dried total lipid was dissolved in concentrated sulphuric acid by keeping in boiling water bath for 10 min. From the prepared total lipid sample, 0.2 ml was taken in a test tube and 5ml of sulphophosphovanillin reagent was added, shaken well and kept undisturbed for 30 minutes. The intensity of red colour was measured at 520 nm in a spectrophotometer.

Estimation of total carotenoids (Quantitative estimation)
Total amount of carotenoid present in the tissue sample was estimated spectrophotometrically as follows. Before quantification, the carotenoid was extracted from the sample by using acetone and methanol and acetone: aeetonitrile solvents. The extracted carotenoid sample was diluted to appropriate volume so as to obtain the optical density value of 0.8 or less. For that the appropriate solvent used for the carotenoid extraction was used. After proper dillution, the optical density was measured at 444nm. The sample was centrifuged and the clear supernatant obtained was used for the measurement of extra cellular carotenoid by taking optical density at 444nm.

Thin layer chromatography analysis of carotenoids
Thin layer chromatographic plates were made by applying slurry made by silica gel of TLC grade and applied over the glass plate. This was dried at 60 c for one hour. The dried plates were pre-activation; base line was drawn on the TLC plate 3cm above the base. Similarly the upper line was marked 15cm away from the base line on the top portion of the TLC plate.3µ1 of condensed carotenoid samples were spotted on the base line of TLC plate at 1cm intervals and then allowed to dry at room temperature. Then the TLC plate was placed in a pre-saturated TLC chamber containing the mobile phase, Acetone and Hexane mixed in the ratio of 90:10 (v/v). The chromatogram was developed in dark environment up to a distance of 15cm mark. Then the plate was taken out and dried for few minutes. The developed and dried chromatogram was stained by using iodine vapour and the developed spots were marked. The distance traveled by each spot in the experimental samples was measured from the base line and relative Rf values were calculated.By comparing the standard Rf values for the chosen mobile phase, the carotenoids present in the samples were identified.

$$\text{Rf} = \frac{\textit{Distance traveled by the solute}}{\textit{Distance traveled by the solvent}}$$

Statistical Analysis
The results obtained in the present study were subjected to relevant statistical analysis explained by Zar (1974) [21].

Results
Biochemical Constituents
Table 1 provide data on the biochemical constituents in the skin tissues of tiger barb (*Puntius tetrazona*) fed with seaweed supplemented diet for different duration (days) 5 and 10 days. The tested biochemical constituents (protein, carbohydrate and lipid) showed much variation between control and experimental groups. The protein content in the skin tissue of control fish was 4.80 ± 0.12 mg/100mg wet weight.

In the experimental fishes, it varied from 4.98 ± 0.14 (days) and 6.58 ± 0.14 (10 days) mg/100 mg wet weight. Similarly, the skin carbohydrate varied from 3.38±0.10 mg/100 mg wet weight in control fish and it ranged from 3.48 ± 0.16 and 3.52 ± 0.12 mg/100 mg wet weight for fishes fed 5 and 10 days. The skin lipid content of control and experimental fish was ranged from 1.52 ± 0.03 to 2.01 ± 0.04 mg/100 mg wet weight.

Total Carotenoid
During experiment, the total carotenoid content in the acetone extract of skin tissue of tiger barb (*Puntius tetrazona*) fish was found to be increased. The total carotenoid content of the control fish skin sample was 2.917 µg/g tissue and which increased during experimentation from 4.0926 µg/g tissue (5 days) and 5.1552 µg/g tissue (10 days).

The total carotenoid content in the methanol skin tissue extract of tiger barb (*Puntius tetrazona*) found to be decreased when compared to acetone extract of skin tissue samples. The total carotenoid content of the control fish was 2.0592 µg/g tissue and which was increased during the experimentation from 2.4972 µg/g tissue and 2.8944 µg/g tissue in 5 days and 10 days feeding experimental fishes.

Similarly, the total carotenoid content in the acetone: acetonitrile extract of skin tissue showed a high carotenoid content in the control fish skin sample was 7.0704 ug/g tissue and it ranged from 12.8892 µg/g tissue and 14.526 µg/g tissue in 5 days and 10 days fed fishes (Table 2).

TLC analysis of carotenoids

In the present study, attempt has also been made to assess the variation in total carotenoids in the selected tissues of koi carp fed with seaweed supplemented diet for different duration (5 and 10 days). Here it was measured in samples extracted with three solvent system.

In the skin tissue of control fish, skin tissue samples extracted with acetone solvent, three carotenoids with Rf value ranged of 0.91, 0.75 and 0.35 were noticed which includes carotene, asteraxanthin ester and one unidentified carotenoids. In the same tissues fed for 5 and 10 days, three and four carotenoids with Rf value range of 0.91, 0.82 and 0.84, 0.91, 0.82 and 0.42 were noticed, which include carotene, asteraxanthin ester, echinenone and unidentified forms.

At the same skin tissue of control fish extracted in methanol solvent system, the carotenoid noticed were carotene (0.91) and asteraxanthin ester (0.75). In 5 days and 10 days fed fishes, two carotenoids were noticed with Rf values 0.91 and 0.40 which includes carotene and unidentfied forms. Similarly, the skin tissue samples extracted by acetone : acetonitrile solvent system, the carotenoids identified were carotene and asteraxanthin ester (control fish), carotene, asteraxanthin ester and echinenone (5 days) and carotene, asteraxanthin ester, echinenone and unidentified forms (Table 3).

Table 1: Changes in biochemical constituents in the various tissues of Koi carp fed with seaweed supplemented diets for different days. Each value is the mean of three individual estimates

Tissues	Duration (days)	Biochemical constituents (mg/100 mg wet tissue)		
		Protein	Carbohydrate	Lipid
Skin	Control(0)	3.379±0.08	3.045±0.07	1.41±0.03
	Control	4.80±0.12	3.38±0.10	1.52±0.03
	5	4.98±0.14	3.48±0.16	1.89±0.03
	10	5.52±0.14	3.52±0.12	2.01±0.04

Table 2: Total carotenoid content in the skin tissue fishes fed with seaweed supplemented diet for different days and extracted in acetone, methanol and acetone: acetonitrile solvent system.

Solvents	Exposure duration (h)	OD	Total carotenoid (µg/g tissues)
Acetone	Control	0.4862	2.9172
	5	0.6821	4.0926
	10	0.8592	5.1552
Methanol	Control	0.3432	2.0592
	5	0.4162	2.4972
	10	0.4824	2.8944
Actone: Acetonitrile	Control	1.1784	7.0704
	5	2.1482	12.8892
	10	2.4210	14.526

Table 3: TLC analysis of carotenoids in the skin tissue of fishes fed with seaweed supplemented diet for different duration and extracted in acetone, methanol and acetone: acetonitrile solvent system

Solvent system	Feeding duration (Days)	Rf Value	Carotenoid
Acetone	Control	0.91	Carotene
		0.75	Asteraxanthin ester
		0.35	Unidentified
	5	0.92	α-carotene
		0.82	Echinenone
		0.84	Asteraxanthin ester
	10	o.84	Asteraxanthin ester
		0.91	α-carotene
		0.82	Echinenone
		0.40	Unidentified
Methanol	Control	0.91	α-carotene
		0.75	Asteraxanthin ester
	5	0.91	a-carotene
		0.40	Unidentified
	10	0.91	α-carotene
		0.36	Unidentified
Acetone: Acetonitrile	Control	0.91	α-carotene
		0.84	Asteraxanthin ester
	5	0.91	α-carotene
		0.84	Asteraxanthin ester
		0.82	Echinenone
	10	0.91	α-carotene
		0.84	Asteraxanthin ester
		0.82	Echinenone
		0.36	Unidentified

Discussion

Carotenoid pigments are biosynthesized by higher plants, algae and certain yeast and bacteria, whereas all animals are believed to depend on the diet for carotenoid supply. Carotenoids are usually present in the integuments of fish, reptiles, amphibians, birds and invertebrates and are often basis for species-specific colouration (Fox, 1976) [8]. Factors such as carotenoid source, diet composition, fish size, growth rate, duration of feeding and metabolic turnover influence the dietary carotenoid uptake and muscle deposition (Torrissen *et al.*, 1989; Sotrebakken and No, 1992) [20, 19].

In the present study, the TLC analysis of carotenoids in the skin tissues tiger barb (*Puntius tetrazona*) fed with seaweed supplemented diet for different duration (days) and extracted in three solvent system shows that with increasing feeding days, the total number of carotenoids increased. For e.g. in the skin tissues extracted by acetone solvent, the fishes fed for 10 days shows the maximum number of carotenoids which includes asteraxanthin ester, carotene, echinenone and one unidentified carotenoid with the Rf values 0.84, 0.91, 0.82 and 0.40 when compared to the control fish.

Moreover due to the high feed intake, nutrient utilization and high nutrient digestibility, deposited nutrients increased. Similar findings were reported in Nile tilapia *Oreochromis niloticus* fed with yeast supplementation (Abdel-Tawab *et al.*, 2006; Abdel-Tawab *et al.*, 2008) [1, 2]. Zheng *et al.* (2009) [22] have also reported increase in the protein content of fish fed with herbal *Origanum heracleoticum* extract incorporated diet. However, Singh *et al.* (2015) [18] reported insignificant difference in whole body carcass composition in the catfish *Clarias gariepinus* fingerlings fed with *Saccharomyces cerevisiae* incorporated in the diet. Similarly, the skin tissues extracted in acetone: acetonitrile solvent system, the same trend was observed. This is because of the supplementation of seaweed in the diet which enhanced the pigmentation. This study was thus designed to compare the various pigments sources (seaweed, chicken intestine, fish meal) in the diets, for their effects on body colouration. Hence an attempt was made to find out the effects of seaweed supplementation on the enhancement body colour of tiger barb (*Puntius tetrazona*).

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