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Mixed algal diet for skin colour enhancement of ornamental fishes

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Abstract

Skin coloration is one of the most important factors which decide the aesthetic, therefore the market value of ornamental fishes. The vibrant pigment profile of different algae (including cyanobacteria) was tested as color elicitor against ornamental fishes. Algal mix incorporated biomass of different taxa like, *Leptolyngbya valderiana, L. tenuis, Arthrospira maxima, Navicula minima* and *Nostoc ellipsosporum* and applied for four types of ornamental fishes such as Golden Gourami (*Trichogaster trichopterus*), Wag-Swordtail (*Xiphophorous hellerii*),Orange Molly (*Poecilia latipinna*) and Pink Zebra (*Danio radio*). 100% algal feed (AF) and 50% supplemented feed (VAF) were tested against Tokyu (commercial feed) as control. The fishes showed good acceptance of VAF compared to the rest with more vibrant coloration and zero mortality. Their pigment study both by spectrophotometric analysis along with HPTLC study showed maximum pigment content in VAF fed fish. The growth performances of the experimental fishes were also found to be better in comparison to control fishes due to nutritious nature of used algal biomass.

Introduction

Ornamental fish rearing is an age old practice because of their high aesthetic value and their ability to survive in an artificial environment. Colored fishes being unable to synthesize their own pigments, depend on exogenous source of pigments like melanins, carotenoids, pteridines and purines (Mukherjee et,al. 2009). Microalgae play an important role as food ingredients for fishes, because of their vibrant pigment profiles. Many microalgae such as *Haematococcus, Arthrospira, Dunaliella, Chlorella, Chlorococcum* have been reported to contain high amount of pigment contents (Borowitzka et al.,1988; Naguib, 2000; Rengel etal.,2000;Guerin etal.,2003).They are rich in chlorophyll, carotenoids and astaxanthin which constitute about 3-5 % of dry algal biomass (Becker 2004).Pigment profile of cyanobacteria and microalgae include α – carotene, ß- carotene, lutein, lutein 5- 6 epoxide, antheraxanthin, zeaxanthin, violaxanthin, neoxanthin, lycopene, canthaxanthin , astaxanthin and many others. Amongst all the pigments, carotenoids play the major role in skin coloration. They are tetra-terpenoid units with a C40 skeleton as their basic molecular structure. Carotenes are pure hydrocarbons while xanthophylls are the derivatives of carotenes with oxygen as functional group (Lohr et al., 1999). Apart from imparting colors to different organisms especially fish, they also act as vital nutrients for enhancing immunity, metabolism and reproduction (Mukherjee et,al. 2009). Therefore they have found wide usage as live feed and as formulated feed in several aquaculture programs (Tanaka et al. 1976; Venkatraman,1980; Webb and Chu 1983; Brown et al. 1989;Avron and Benemann,1992; Lee,1997; Yamaguchi,1997; Gouveia et al. 1997; Tacon 1981; Raymundo et al. 2005). Along with the pigment content, high protein, carbohydrate and vitamin content of algae also make them nutritionally more potent and popular organism in aquaculture based animal rearing.

Several studies have shown the usage of different algae such as *Porphyridium, Isochrysis, Pavlova Chaetoceros, Gracillaria, Palmaria and Arthrospira* as efficient color elicitors in cichlid fish, rainbow trout , fish larvae, bivalve mollusks and several

gastropods (Kop and Durmaz 2008; O'Connor et al., 1997;Neori and Shpigel 1998; Corazani and Illanes 1998; Boarder and Shpigel 2001 ;Gupta et al., 2007;Nandeesha et al., 2001; Nickell and Bromage 1998;Sudaporn et al., 2010.) Effects of *Isochrysis* on pigmentation, growth and survival of black tiger prawn *Penaeus monodon* post larvae has also been reported by Chih-Hung et.al.(2001). Along with the increase in the pigment content, a decrease in the mortality rate in different varieties of fishes such as Tetra, Cichlids, Nile tilapia, Rainbow trout have also been reported on feeding with *Ulva* , *Cytoseira* or *Chlorella* (Deventor and Heckman1996; Gouveia 1997;Gouroy et al.,2007).

But the main constraint of microalgal use in aquaculture is their high cost of production, which limits the use of microalgal biomass. The present group has already reported the use of non-conventional algal genera in the form of live and formulated fish feed as efficient color elicitors for Gold fish (Khatoon et al., 2010a,b) and *Hemmigramus caudavitatus* (Mukherjee et al.,2013) in a cost effective way. In the present investigation different genera of commonly occurring micro-algae viz *Leptolyngbya valderiana* (Gomont) Anagnostidis & Komarek, *Leptolyngbya tenuis* (Gomont) Anagnostidis & Komarek, *Arthrospira maxima* Setchell and N.L. Gardener, *Navicula minima* Gronow and *Nostoc ellipsosporum* N. L. Gardener were selected and two types of feeds were formulated by total and 50% replacement of market feed. The formulated feed were applied to four types of colored fishes such as, Golden Gourami (*Trichogaster trichopterus*) Pallas , Wag-Swordtail (*Xiphophorous hellerii*) Heckel,Orange Molly (*Poecilia latipinna*) Valenciennes and Pink Zebra (*Danio rerio*) Paull for 30 days. Their growth performances were studied and pigment contents were analyzed using HPTLC method.

Materials and Methods

Collection and Culturing Of Algae:

The microalgal strains, *Leptolyngbya valderiana, Leptolyngbya tenuis, Arthrospira maxima, Navicula minima* and *Nostoc ellipsosporum* were cultured in an unialgal batch culture mode at laboratory condition (Temperature 20-25^{0}C,16:8 light dark period, light intensity- 1500 – 1700 lux, pH 7.5) using ASN(III) media(*Leptolyngbya valderiana, Leptolyngbya tenuis*), ASN(III) media + silicate (*Navicula minima)*, Zarrouk's media(*Spirulina platensis*) and BG11(*Nostoc ellipsosporum*) media.

Media Composition:

i. ASNIII : 1 L medium contains, NaCl- 25g, $MgCl_2$.2g, KCl- 0.5g, $NaNO_3$.0.75g, $K_2HPO_4.3H_2O$- 0.02g, $MgSO_4.7H_2O$ -3.5g, $CaCl_2$.0.5g, EDTA- 0.0005g, Na_2CO_3 .0.02g.

ii. ASNIII media + Sodium meta-silicate (30mg/l)

iii. Zarrouk's media :
1Lmedium contains, $NaHCO_3$-13.61g, Na_2CO_3-4.03g, K_2HPO_4 - 0.5g; $NaNO_3$- 2.50g, K_2SO_4 - 1g, NaCl-1g , $MgSO_4.7H_2O$-0.20g., $CaCl_2.2H_2O$-0.04g, $FeSO_4.7H_2O$- 0.01g, EDTA-0.08g, micronutrient-5ml.

iv. BG11 media: 1 L medium contains $NaNO_3$ - 1.5g, K_2HPO_2-2g, $MgSO_4.7H_2O$ - 0.075g, $CaCl_2.2H_2O$-0.036g, Citric acid-0.006g, Ferric ammonium citrate-0.006g,Na_2CO_3-0.02g, Trace metal mix-1ml.

Diet Formulation:

Two types of diets were formulated (Diet 2 and 3):

Diet 1: Market available conventional feed (Tokyu) considered as control feed (CF)

Diet 2: Algal feed (AF) – Different taxa in following proportion were mixed - *Leptolyngbya valderiana :Leptolyngbya tenuis: Arthrospira maxima: Nostoc ellipsosporum*: *Navicula minima* :: 40:20:25:5:10 respectively as total or 100% replacement.

Diet 3: Value added feed (VAF) – A mixture was prepared with 50% commercial feed with 50% algal biomass supplementation

Feed Preparation:

During feed preparation program cyanobacteria along with the algal biomass were dried, weighed, crushed and were thoroughly mixed with commercial binder (wheat flour) to produce a homogenous mixture. Then 2 mm diameter pellets were made out of the dough. The pellets were then dried in hot air oven at 60°C for 3-4 hours. After drying, the pellets were packed in an airtight bag and stored in freezer for further use.

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Collection and Rearing of Experimental Fish:

A static indoor rearing system was used for conducting the feeding trial. Four types of ornamental fishes (Golden Gourami, Wag-Swordtail, Orange Molly and Pink Zebra) were collected from a local vendor. The total number of fishes was 1,800 (450 of each type) and they were divided and acclimated in 9 large aquaria with artificial aeration and continuous flow system for seven days under natural condition. During this tenure they were fed with commercial feed.

Experimentation:

For experimentation, 50 fishes of each type were randomly stocked in three tanks with continuous artificial aeration and flow systems. Three different types of diet were given to the respective tanks. Each diet was given at the rate of 75% of the body weight of the fish. The experimentation was carried out for a period of 4 weeks. Throughout the tenure the water in 3 tanks were replenished thrice in a week to avoid accumulation of unutilized food and metabolic wastes at a specific hour of the day. The left over feed were collected at time of cleaning, then dried and weighed to see the acceptability of algae as their food. All the data were collected after 15 and 30 days of experimentation. Growth performances and pigment analysis were considered after 30 days only (when maximum changes were observed).

Pigment Analysis:

A spectrophotometric analysis for quantitative estimation of pigments (carotenoid, astaxanthin) from algal biomass, feed and the fishes were carried out, using different standard protocols like Sadasivam and Manickam1996; Sachindra and Mahendrakar2005; respectively.

Qualitative Estimation of Carotenoid by HPTLC (High Performance Thin Layer Chromatography) (CAMAG):

This method was used to determine the components of carotenoids present in the selected cyanobacteria and algal specimen and experimental fish. Here the cyanobacteria and algal biomass as well as the fish were crushed in acetone and an aliquot of 20µl carotenoid extract was applied to the TLC plate along with the standards (astaxanthin and β- carotene) and the chromatogram was developed in petroleum ether: acetone in the ratio of 7:3 as running solvent. Then the plate was analyzed with the help of a scanner.

Growth Parameter Analysis of Fish:

Initial and final body weight of the fish were analyzed and other growth parameters including nutrient utilization of the experimental fish were evaluated by the following formulae (Becker et al.,1999; Siddhuraju and Becker 2003):

1. Body weight gain (BWG) = [{Final body weight (g) - Initial body weight (g)}/Initial body weight]

2. Specific growth rate (SGR) = [In Final body weight (g) – In Initial body weight(g) / Number of days] x 100

Body weight, total length and breadth were also measured according to (Holick et al.,1989 and Elvira et al., 2000). The amount of feed intake per day was also recorded.

Statistical Analysis:

One way ANOVA technique was used to compare the pigment content of the fish at initial and final stage. The significance of difference between the mean was determined by Duncun's multiple range test ($P < 0.05$) using SPSS for windows (10.0) (Duncun 1995).

Results

Growth Performances of Fish

Growth performances of fingerlings of experimental fishes fed on three different diets (CF, AF and VAF) at initial stage and after 30 days of treatment are shown in Table 1.

The results indicated an increase in growth performances in relation to Body weight, Body length and Specific growth rate for VAF fed fish with respect to that of CF and AF fed fishes (Table 1). Maximum growth was found in case of VAF fed Pink Zebra followed by Golden Gourami, Orange molly and Wag Swordtail. An increase of 1.02 – 1.7 folds for SGR% was observed in case of VAF fed fishes (all 4 types) when compared to the AF fed and CF fed fishes.

Table 1: Table showing growth performances of different fishes fed with control and experimental diet

FISH TYPES	FEED TYPES	TIME	BODY WEIGHT (g)	BODY LENGTH (cm)	SPECIFIC GROWTH RATE (%)
GOLDEN GOURAMI ***(Trichogaster trichopterus)***	FEED 1	0 WEEK 4TH WEEK	1.26 ±0.04 2.26±0.02	4.4±0.04 5.2±0.04	3.33± 0.25
	FEED 2	0 WEEK 4TH WEEK	1.26 ±0.04 2.40±0.05	4.4±0.04 5.4±0.03	3.8± 0.30
	FEED 3	0 WEEK 4TH WEEK	1.26 ±0.04 2.51±0.05	4.4±0.04 5.5±0.05	4.16± 0.29
WAG-SWORDTAIL (*Xiphophorous hellerii)*	FEED 1	0 WEEK 4TH WEEK	0.704±0.04 1.16±0.13	3.7±0.31 4.1±0.11	1.52± 0.05
	FEED 2	0 WEEK 4TH WEEK	0.704±0.04 1.27±0.17	3.7±0.31 4.2±0.19	1.88± 0.04
	FEED 3	0 WEEK 4TH WEEK	0.704±0.04 1.40±0.05	3.7±0.31 4.3±0.21	2.32± 0.06
ORANGE MOLLY (*Poecilia latipinna)*	FEED 1	0 WEEK 4TH WEEK	1.01±0.02 1.46±0.02	3.9±0.11 4.3±0.17	1.5± 0.11
	FEED 2	0 WEEK 4TH WEEK	1.01±0.02 1.78±0.04	3.9±0.11 5.0±0.17	2.57± 0.05
	FEED 3	0 WEEK 4TH WEEK	1.01±0.02 1.98±0.03	3.9±0.11 5.2±0.11	3.22± 0.01
Pink Zebra ***(Eupalaestrus campestratus)***	FEED 1	0 WEEK 4TH WEEK	0.241±0.012 0.421±0.012	2.7±0.11 3.6±0.23	0.60± 0.057
	FEED 2	0 WEEK 4TH WEEK	0.241±0.012 0.447±0.008	2.7±0.11 3.8±0.17	0.68± 0.023
	FEED 3	0 WEEK 4TH WEEK	0.241±0.012 0.478±0.007	2.7±0.11 4.0±0.28	0.79± 0.034

Pigment Analysis of Algae and Experimental Fish

The spectrophotometric and the chromatographic pigment analysis of the cyanobacteria and algal specimens showed a good content of pigments in all the samples. The study showed maximum carotenoid content in *Arthrospira platensis* followed by *Leptolyngbya tenuis* and *Leptolyngbya valderiana* (fig 1a.and plate.1.). The astaxanthin content was further found to be more in *Navicula minima* followed by *L. tenuis* (fig.1b.and plate.1.).

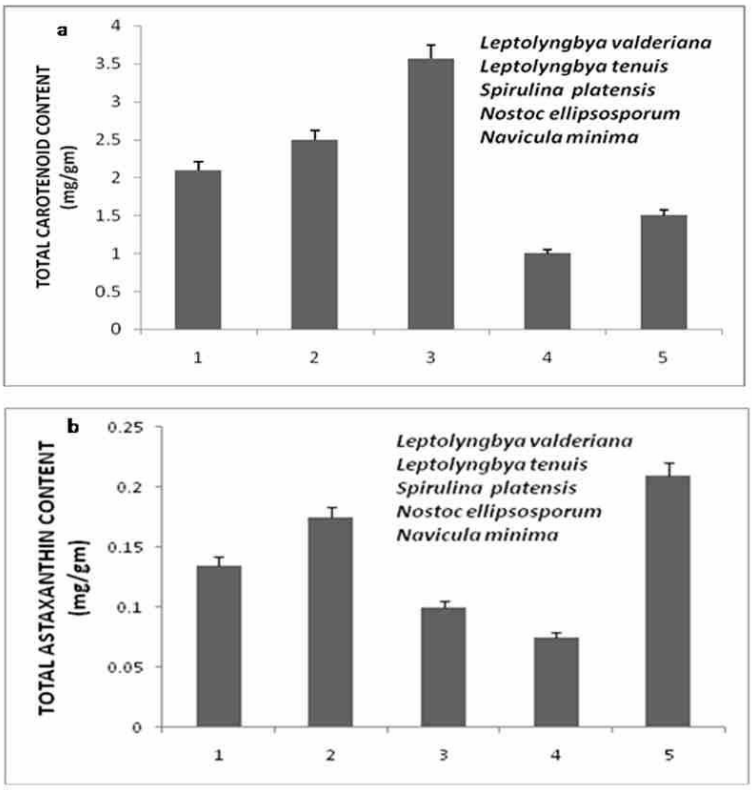


Figure 1: Graphical representation of the pigment contents a. Carotenoid, b. Astaxanthin of selected algae.

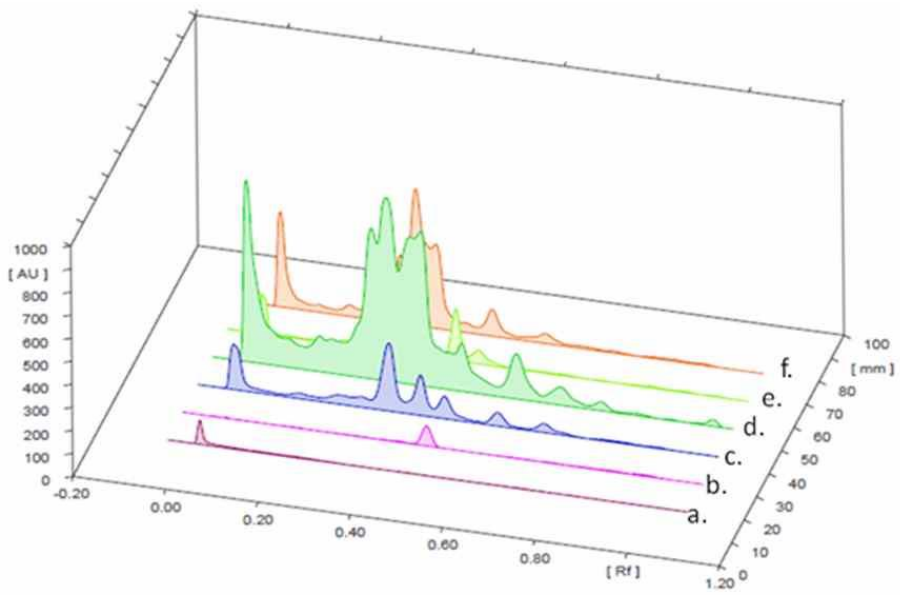


Plate 1: HPTLC study of algal samples showing a. Standard β-carotene, b. Standard Astaxanthin, c.*Leptolyngbya valderiana,* d.*Leptolyngbya tenuis*, e.*Spirulina platensis*, f.*Nostoc ellipsosporum,* g. *Navicula minima.*

Both the chromatographic and spectrophotometric study (fig 2. and plate.2.) of the pigment content of the fishes showed that the ornamental fishes responded more positively to value added diet as compared to the total algal diet and commercial feed. In this

experimentation VAF and AF fed Golden gourami showed 2.6 and 1.86 folds ($p< 0.05$) more carotenoid content compared to the control fish respectively. VAF and AF fed Wag sword tail were found to show 1.66 and 1.33 folds ($p< 0.05$) more carotenoid content as compared to control fish respectively. An increase in 1.9 and 1.66 folds ($p< 0.05$) was displayed by VAF and AF fed Orange Molly and 2.5 and 2 folds ($p< 0.05$) by VAF and AF Pink Zebra respectively when compared with the carotenoid content of commercial feed fed fish(fig 2a, plate2a,b,c).

Along with the carotenoid content, the astaxanthin content of the experimental fishes were also measured. VAF fed Wag sword tail showed the maximum content of astaxanthin in comparison to the control set up as well as the 100% algal supplemented setup (1.23 , 3.2 folds more respectively). 1.23 And 2.3 folds more astaxanthin content were found in case of both VAF fed Orange Molly and Pink Zebra fishes as compared to the AF fed and CF fed fishes. Similar results were also found in case of Golden gourami. These results have been represented in the figure 2b and plate 2d and 2e.

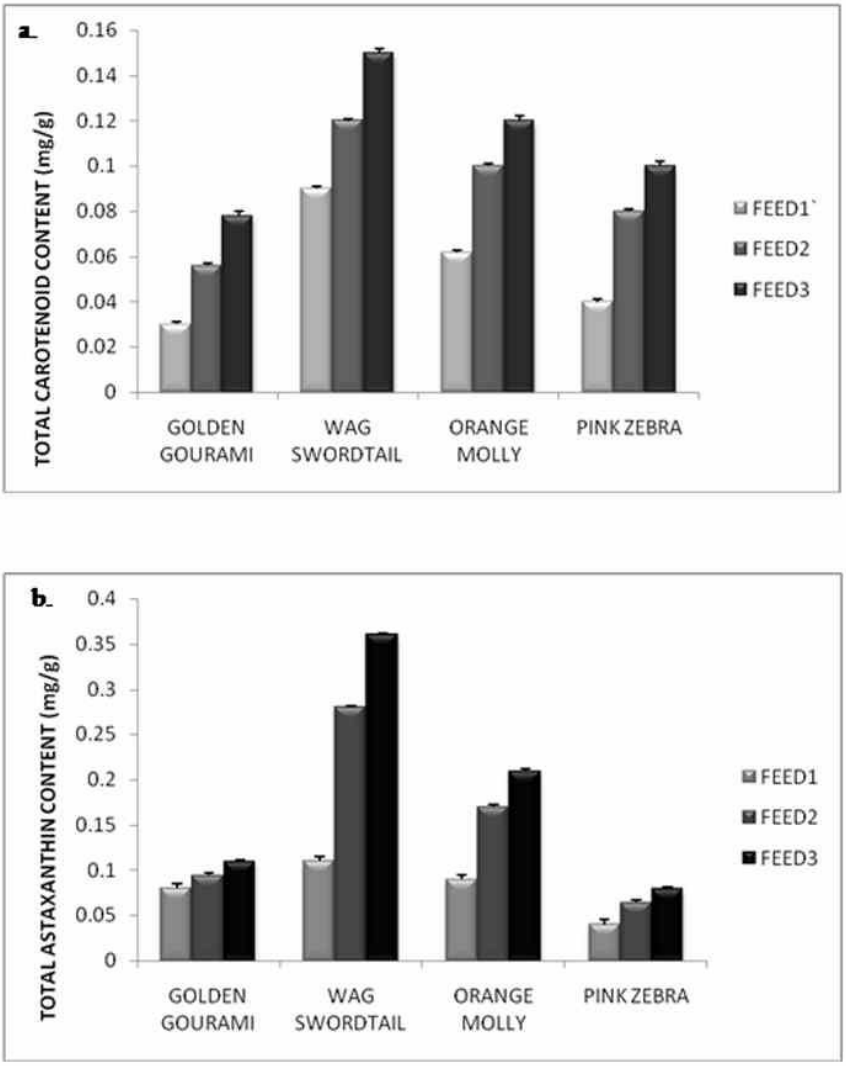
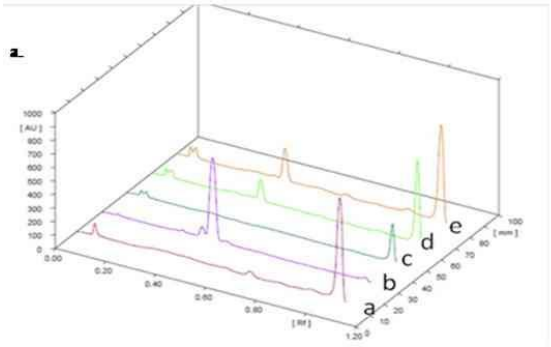
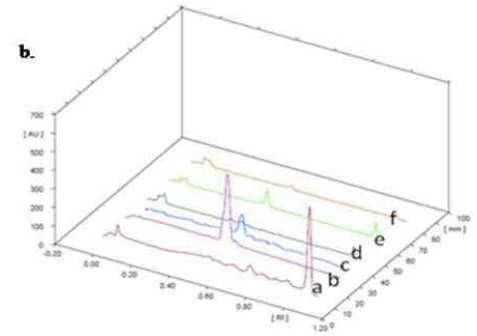


Figure 2: The graphical representations show a. Total carotenoid and b. Total astaxanthin content of the experimental fishes fed with different types of fish feed.

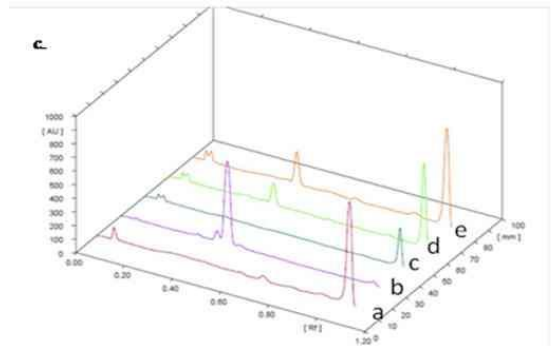


HPTLC plate showing peaks of β carotene and Astaxanthin in a. Standard β-carotene,b. Standard astaxanthin, c. Fish Wag-sword tail, d. Fish Orange Molly, e. Fish Pink Zebra, f. Fish Golden Gourami. The peaks of the lanes c-f show very low amount of β-carotene and astaxanthin in 0 days respectively from front to back



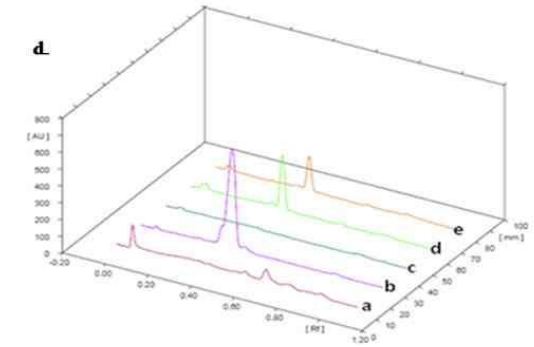
HPTLC plate showing peaks of β carotene and Astaxanthin in a. Standard β-carotene,b. Standard astaxanthin, c. Commercial feed fed Golden Gourami, d.AF fed Golden Gourami e. VAF fed Golden Gourami respectively from front to back after 30 days.

The peak of lane d and e. show higher pigment content than the control



HPTLC plate showing peaks of β carotene and Astaxanthin in a. Standard β-carotene,b. Standard astaxanthin, c. Commercial feed fed Wag-sword tail, d.AF fed Wag swordtail e. VAF fed Wag sword tail respectively from front to back after 30 days.

The peak of lane d and e. show higher pigment content than the control



HPTLC plate showing peaks of β carotene and Astaxanthin in a. Standard β-carotene,b. Standard astaxanthin, c. Commercial feed fed Orange Molly, d.VAF fed Orange Molly e.AF fed Orange Molly respectively from front to back after 30 days.

The peak of lane d and e. show higher pigment content than the control

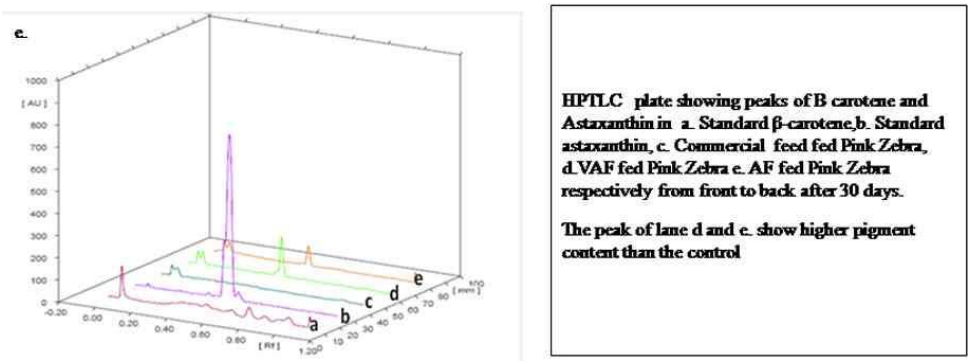


Plate 2: Chromatographic representation of b- carotene and astaxanthin content in each experimental fish types fed with different feed with respect to the authentic pigments.

Discussion

Algae form an important part of the food web. They are the primary producers and enter into the fish food chain via the zooplankton population. Therefore their incorporation within the fish diet would be highly beneficial. But presently several surveys in different parts of the world have shown that their usage in aquaculture is still at infancy due to high cost of production. Thus to promote their use we need to find a cost effective beneficial mixed algal formulated diet. In this aspect our motto was to use different algae with high pigment contents and other biochemical profiles, in formulating a potential algae based fish feed. Since unialgal diet becomes costly therefore a cost effective mixed algal diet has been formulated and their performances as fish pigment elicitors have been noted (*Leptolyngbya valderiana, Leptolyngbya tenuis, Arthrospira maxima, Navicula minima* and *Nostoc ellipsosporum* mix *was* tested on Golden Gourami (*Trichogaster trichopterus*), Wag-Swordtail (*Xiphophorous hellerii*),Orange Molly (*Poecilia latipinna*) and Pink Zebra (*Danio rerio*)}

In this experimentation, results have revealed that maximum skin coloration have been induced by the value added diet as compared to the other two diets such as AF and CF, which was maximum in Wag swordtail. This is in full agreement with the earlier works of present groups where a mixture of *Phormidium valderianum, Nostoc and Navicula* increased the pigment content in, *Hemmigrammus caudavitatus*, prawn and Gold fish (Mukherjee et al., 2013 and Khatoon et al., 2009). Similar results were also shown by works of other authors in *Haematococcus* fed shrimps (Arredondo-Figueroa, 2003; Boonyaratpalin et al., 2001; Yamada et al., 1990) . According to the work of Khatoon et. al., 2010, VAF fed Goldfish (*Carassius auratus*) showed about7.2 folds increase in carotenoid content as compared to control fed fish. Similar results were also shown by VAF fed prawn over control setup in the work of Khatoon et al.(2009). Even though maximum pigment content has been found in algae based feed as compared to the value added and commercial feed, but maximum utilization of the value added feed by the fishes led to maximum coloration compared to algae based feed.

Apart from the skin coloration an increase in the body weight gain, specific growth rate, and body length showed a positive correlation with the external supplementation of algal pigments. Similar results of other authors showed increase in body weight as well as specific growth rate percentage in tetra, gold fish, *Oreochromis niloticus* on treatment with microalgae based feed prepared with different cyanobacteria and algae such as *Phormidium, Arthrospira, Chlorella, Scenedesmus.*(Mukherjee et al., 2013; Khatoon et al.,2010a; Dawah et al., 2002b;Nandeesha et al.,1998 and Badaway et al.,2008)

Generally the carotene obtained from algal diet are converted to astaxanthin, which acts as a potent antioxidant improving the immunity of the fishes as observed in Tetras and Cichlids, Nile Tilapia etc. fed on *Ulva* , *Cystoseira* and rhodophycean members. Studies of various workers have revealed that both β carotene as well as astaxanthin play an efficient role in removing free radicals, enhance the resistance power against fungal diseases and also improves hepatopancreatic function , provides protection to the cells against photoxidation, as well as protects the polyunsaturated fatty acids and cholesterol content of the organism (Britton 1995;Chien et al., 2003;Supamattayaa et al.,2005; Pan et al., 2003; Mcnulty et al., 2008; Palozz et al., 2008). A significant positive correlation between pigment content of prawn and their survival rate also suggested that both β carotene and astaxanthin have a positive role in improving the immunity of the organism (Chien & Jeng 1992; Passos 2006 and Boonyaratpalin et al., 2001).In present investigation also the mortality rate was found to be negligible.

Conclusion

From the overall study, it can be concluded that mixed algal diet, due to its high pigment content and good nutrient parameter can easily be used as feed ingredient for ornamental fish to achieve better results. Apart from acting as good pigment elicitors they would efficiently increase fish immunity by providing protection against bacterial and fungal diseases. Thus this would help to increase the market value of the ornamental fish in near future. Thus this cheap and potent mixed algal diet may successfully replace other high value unialgal biomass as fish feed ingredients.

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