Research Article

J. Algal Biomass Utln. 2013, 4 (3): 59–65 Cyanobacteria as Elicitor of Pigment in Ornamental Fish
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Cyanobacteria as Elicitor of Pigment in Ornamental Fish *Hemigrammus caudovittatus* (Buenos Aires Tetra)

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Abstract

A feeding experiment was carried out to evaluate the efficacy of the cyanobacterial member *Phormidium valderianum* Gomont as a color elicitor for *Hemigrammus caudovittatus* Eigenmann an ornamental fish. Biochemical analysis of the cyanobacterial biomass with high protein (25. 5%) and carotenoid value (1.78 mg/gm) indicated the genus as a potential feed ingredient. Cyanobacterial biomass was used as total and partial replacement of feed ingredient and compared to the commercial feed. Significant enhancement in carotenoid content (1.81 folds) were recorded in case of 50% feed replacement, together with other increased growth parameters like protein content ,growth performance, feed conversion ratio, protein efficiency ratio and protein productive value. Thereby it was evident that incorporation of cyanobacteria in color fish diet would not only increase their market value but would also enhance their growth performances. The changes in the algae based diet fed fish were found to be statistically significant. Principal component analyses among all the variables of experimental fish were also performed.

Key Words: Cyanobacteria, fish feed, biochemical parameters, principal component analyses (PCA).

Introduction

The brilliant coloration of fish has always allured people for keeping it as pet due to its high aesthetic value since the Ming Dynasty (1368-1644). Presently, the market of the colored fish industry has been estimated to be US$ 327 million (2007). The coloration of fish is due to synthesis and secretion of pigments by specialized cells present below their scales, called the chromatophores. Since fish cannot synthesize their own color, therefore they need to depend on their diet for coloration. Thus, their diet must include the ingredients which would preferably enhance their coloration.

Several studies have shown that microalgae such as, *Chlorella vulgaris*, *Dunaliella salina*, *Haematococcus pluvialis* and *Arthrospira maxima*, to be potent carotenoid producers thereby enhancing the skin coloration of fish (Gupta et al.2007). Microalgae also provide better health to the fish because of its high protein and carbohydrate content (Becker 2004). Different algal genera such as *Spirulina , Chlorella, Scenedesmus* etc have a crude protein content from 40% to 50% , total carbohydrate content from 25%-60% and a considerable amount of β- carotene content. These biochemical compositions of microalgae, being the indices of nutritional values have allowed them to be used as efficient feed ingredients in several aquacultural programs (Tanaka et al. 1976; Tacon 1981; Gouveia et al. 1997; Raymundo et al. 2005 ; Brown et al. 1989; Webb and Chu 1983). Carotenoids on one hand act as skin color elicitors, on the other hand they also provide immunity to the fish because of their inherent antioxidant property. Kop and Durmaz (2008) used *Porphyridium cruentum* as color elicitor in cichlid fish while *Isochrysis, Pavlova lutheri* and *Chaetoceros calcitrans* were used in larval culture of bivalve mollusks by other workers (O'Connor et al.1998).

Thus, due to the aforesaid reasons microalgae can be used in several aquacultural programs as efficient feed ingredient. But the major constrain of using microalgae in fishing farm is their high production cost (US$300-3000 per kg). Thus their use in aquaculture industry is still at its infancy. Therefore, in search for a comparatively cheaper algal resource the cyanobacterial genus *Phormidium valderianum* was tested for its easy availability in tropical coast line and cheap production cost. Furthermore, only a short period of time is required for the mass *Phormidium* production of this cyanobacterial biomass.

Phormidium spp. is quite common in Indian coast (Thajuddin and Subramaniam 2005) and also reported in West Bengal by the present group (Chattopadhyay and Pal 1995). The strain was collected from coastal areas and used as feed. After proper biochemical analysis feeds were formulated by total and 50% replacement of market available feed by cyanobacteria by and applied to the good quality ornamental fish *Hemigrammus caudovittatus* for 30 days in the present investigation.

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Materials and Methods

Collection and culturing of algae

Cyanobacterial genus, *Phormidium valderianum* was collected from south-eastern part of Indian Sundarbans and cultured in an unialgal batch culture mode both at laboratory condition (Temperature 20-25C, pH 7.5) using ASN(III) media (25gms/L NaCl, 2gms/L$MgCl_2$, 0.5g/L KCl,0.75g/L $NaNo_3$,0.02g/L $K_2HPO_4.3H_2O$, 3.5g/L $MgSO_4.7H_2O$, 0.5g/L $CaCl_2$, 0.0005g/L EDTA, 0.02g/L Na_2CO_3) and in open tank culture using simplified media .

Biochemical analysis of Cyanobacterial biomass

Biochemical parameters like proteins, carbohydrate and carotenoids of healthy growing biomass were analyzed, using different standard protocols like Lowry et al.1951; Hodge and Hofreiter 1962; Sachindra and Mahendrakar 2005.

Diet formulation

Three types of diets were used –

Diet 1 – Market available commercial feed considered as control feed (CF).

Diet 2 – Algal feed (AF) -only *Phormidium* biomass as a total or 100% replacement.

Diet 3 – Value added feed (VAF) - 50% commercial feed with 50% algal biomass Supplementation.

Feed Preparation

The cyanobacterial biomass was dried, weighed, crushed and was thoroughly mixed with commercial binder 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 60C for 3-4 hours. After drying, the pellets were packed in an airtight bag and stored in freezer for further use.

Biochemical analysis of feed

Biochemical parameters of the feed like protein, carbohydrate and carotenoid were analyzed using standards as mentioned earlier.

Collection and rearing of experimental fish

A static indoor rearing system was used for conducting the feeding experiment. About 150-170 fingerlings of *Hemigrammus caudovitattus* were purchased from local vendor. The fish were then acclimated in large glass aquaria with artificial aeration for seven days under laboratory condition. During this period commercial feed was provided to them.

Experimentation

About 50 fingerlings were randomly stocked in three tanks each and three types of above mentioned diets were given to the respective tanks. During the trial program water quality and temperature of each tank was thoroughly monitored. To maintain the water quality regular water change in the tanks were performed during noon. The feed were given at an interval of 24 hours at noon at the ratio of 75% of the total body weight of fish. 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 biochemical parameters were considered after 30 days only (as maximum changes were observed). For Principal component analysis of variables both 15 days and 30 days data were considered.

Analysis of growth and biochemical parameters 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)-

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1. Body weight gain (BWG) percentage = [{final body weight(g) - Initial body weight(g)}/Initial body weight] x 100
2. Specific growth rate (SGR) = [In Final body weight(g) – In Initial body weight(g) / Number of days] x 100

3. Feed conversion ratio (FCR) = Dry feed fed (g)/Live body weight gain (g)

4. Protein efficiency ratio (PER) = Body weight gain/ Crude Protein fed (g) x 100

5. Protein productive value (PPV) = Gain in fish body protein / Crude protein.

Biochemical parameters of the fish like protein, carbohydrate and carotenoid were analyzed using standards as mentioned earlier.

Quantitative Estimation of Carotenoid in Cyanobacteria and Fish

A spectrophotometric analysis of both the cyanobacteria and the fish was carried out for quantitative estimation of their carotenoid content. The biomass as well as the fish flesh was crushed in acetone. The supernatant was collected and estimated at a wavelength of 450nm.

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

This method was used for determining the components of carotenoids present in experimental fish. Here the fish was crushed in acetone and an aliquot of 20µl carotenoid extract was applied to the TLC plate along with the authentic (astaxanthin and β- carotene) and was run in Petroleum Ether along with acetone in the ratio of 7:3 as running solvent. Then the plate was analyzed with the help of a scanner.

Statistical Analysis

Using one way ANOVA technique the biochemical parameters such as protein, carbohydrate and carotenoid content of the feed and the experimental fish were compared. Further analysis was also performed amongst the biochemical parameters of the experimental fish. The significance of difference between the means was determined by Duncun's multiple range test ($P < 0.05$) using SPSS for wjndows (10.0) (Duncun 1995).

Correlation matrix among the variables was also developed along with Principal Component Analysis.

Results

In the present experimentation the efficiency of *Phormidium valderianum* as fish feed had been evaluated using *Hemigrammus caudovittatus* as the experimental species. The biochemical parameters of *Phormidium valderianum* are represented in Fig.1a,b,c showing 255.3mg/gm protein, 32.88mg/gm carbohydrate and 1.78 mg/gm carotenoid content of the experimental biomass.

The biochemical analysis of the fish feed showed that the value added feed (VAF) had 1.17 fold and 1.43 fold more protein as compared to market available commercial feed as well and algal feed (AF) respectively. Value added feed also showed (1.15 and 1.06 folds) more carbohydrate content and 2.76 and 1.33 folds increased carotenoid content with respect to CF and AF respectively (Fig. 1a,b,c). Therefore the value added feed was found to be more efficient than the other two feeds.

Growth performance of fish

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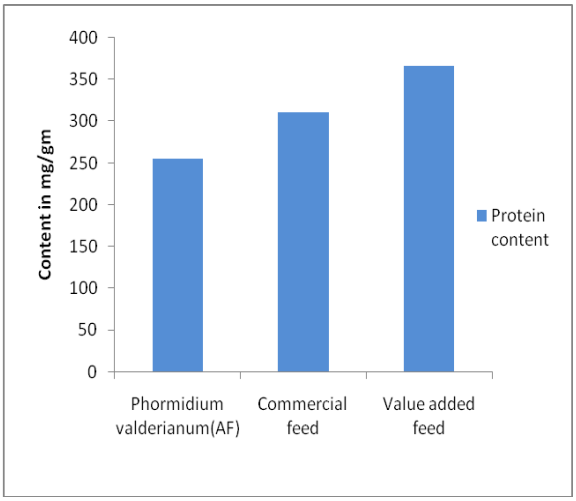

Figure 1a. Total protein content of control and experimental feeds.

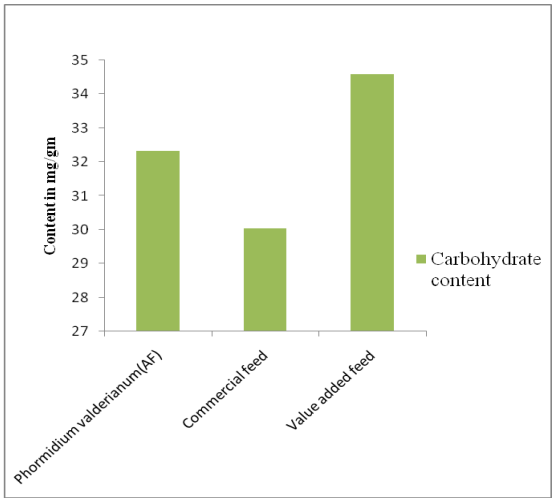

Figure 1b. Total carbohydrate content of control and experimental feeds.

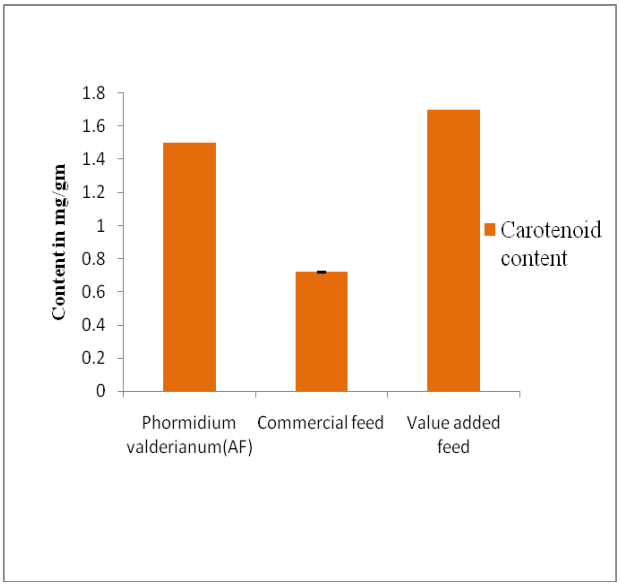

Figure 1c. Total carotenoid content of control and experimental feeds.

Growth performance of fingerlings of *Hemigrammus caudovittatus* fed on experimental (CF, AF and VAF) diets after 30 days of treatment are shown in Table 1.

The results showed that the rate of increase in growth performances in relation to SGR % and BWG % for VAF fed fish was more with respect to that of CF and AF fed fish (Table 1). Similarly an increase by 1.16 fold for SGR% and 1.15 fold for BWG% were observed when fish was fed with VAF, whereas 1.5 fold decreases in SGR % and for BWG% for AF (100% replacement) fed fish were recorded . These results indicated that VAF with high protein and carbohydrate value was more effective for fish growth than CF and AF (Figs.1 a, b). The protein efficiency ratio was also found to be better in case of VAF fed fish as compared to that of AF as shown in Table 1.

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Table 1. This represents the growth parameters of the fish fed with control and experimental feeds after 30 days of rearing.

Growth Parameters	30 days		
	Commercial feed (CF)	Algal feed (AF)	Value added feed (VAF)
Body weight gain (%)	34.28	22.85	40
Specefic growth rate (%)	0.4	0.266	0.46
Feed conversion ratio	0.736	0.568	0.625
Protein efficiency ratio (%)	0.109	0.087	0.109
Protein productive value	0.301	0.28	0.77

Further analysis by ANNOVA test showed a significant increase ($p < 0.05$) of protein content of VAF fed fish by 2.2 folds and 1.15 fold decrease for AF fed fishes compared to controlled one(Fig. 2a). The protein productivity was also found to be more in case of VAF as compared to the rest as shown in Table 1. The carbohydrate content of VAF fed fish (Fig.2b) was found to increase significantly ($p < 0.05$) by 1.23 fold more as compared to the control fish. Therefore the protein and carbohydrate content were enhanced by 50% replacement of feed ingredients by cyanobacterial biomass. Therefore, *Phormidium* valderianum was found to be favorable as the dietary supplement of the experimental fish.

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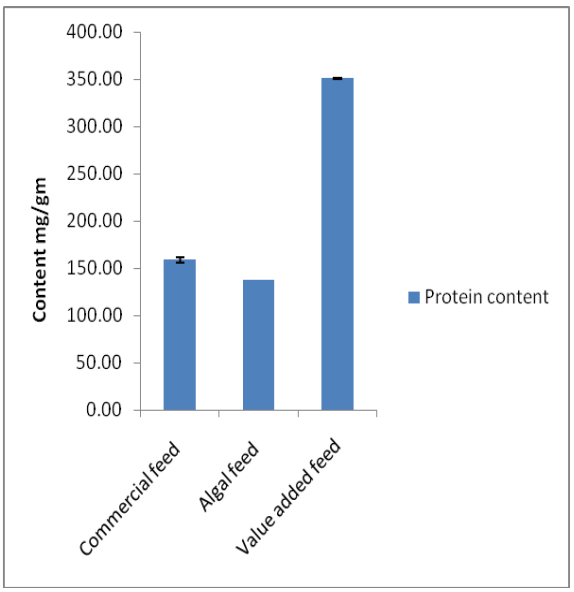

Figure 2a. Variation in protein content of fish fed with control and experimental feeds after 30 days of rearing.

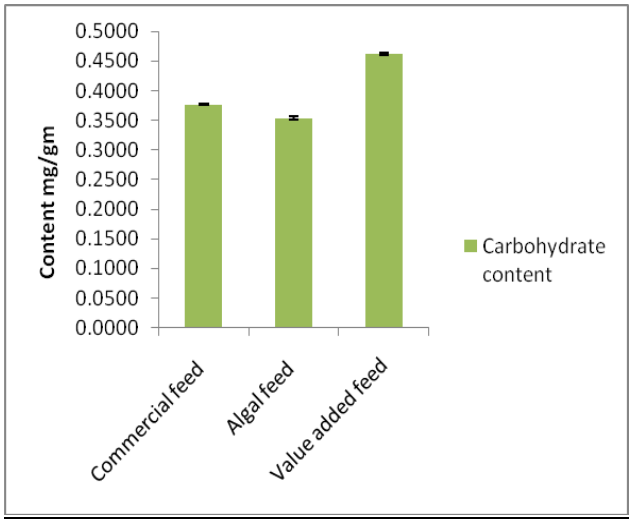

Figure 2b. Variation in carbohydrate content of fish fed with control and experimental feeds after 30 days of rearing.

Analysis of pigments

By analyzing the pigment content, it was observed that the fish fed with VAF showed a significant increase ($p< 0.05$) of 1.81 fold and 1.83 fold in carotenoid content as compared with the fish fed with CF as well as with the AF respectively as represented in Fig.2c.

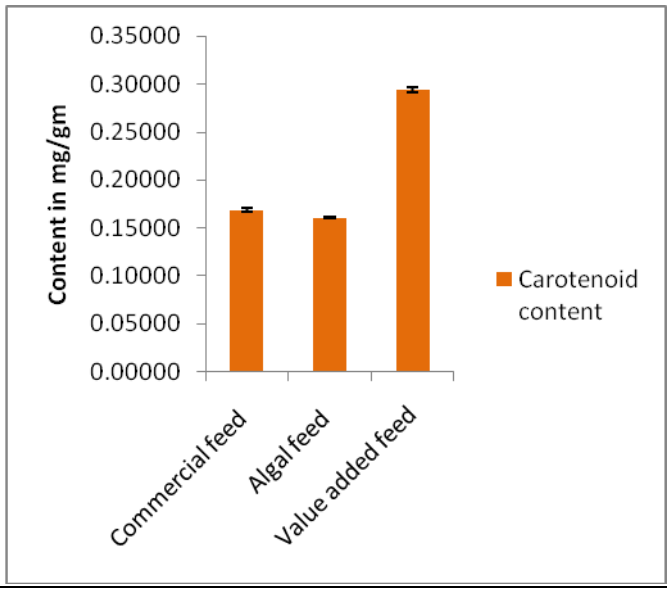

Figure 2c. Variation in carotenoid content of fish fed with control and experimental feeds after 30 days of rearing.

The mortality rate for fish fed with VAF was also found to be lower as compared to the other two sets. About 80% fish survived in case of algae fed fish and 88% lived in case of commercial fed fish whereas almost 92-94% fish survived in case

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of value added fed fish due to accumulation of more carotenoid content. Further, more coloration was also observed in the operculum and head regions of VAF fed fish as shown in Plate 2.

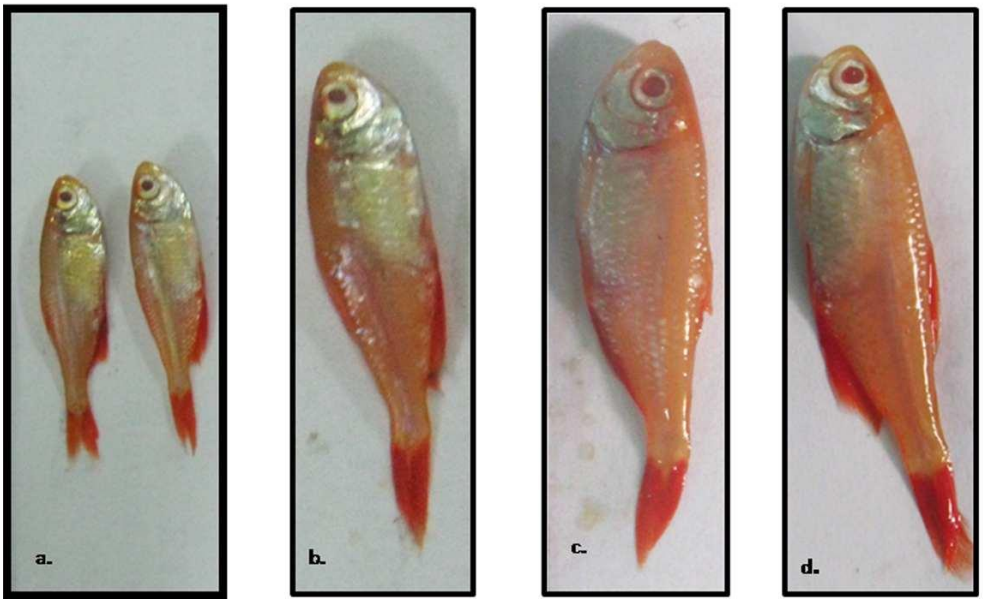

Plate 2 a. Control Fish (0 days); b. Experimental fish fed with commercial feed for 30 days.

c. Experimental fish fed with algal feed for 30 days. d. Experimental fish fed with \value added feed for 30 days

The HPTLC study of the carotenoid content of fish distinctly showed the presence of astaxanthin and B-carotene for all the three feed fed fish. In addition to the above a red fluorescent band was also seen in the fish fed with algal feed as shown in the HPTLC (Plate 1).

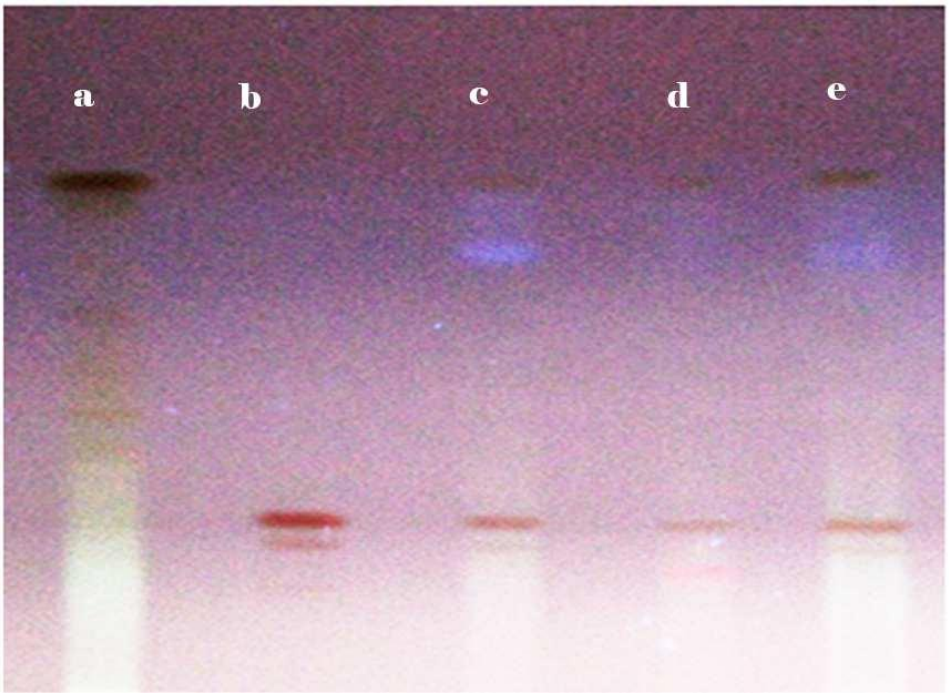

Plate 1. HPTLC Plate showing the presense of Astaxanthin and β – carotene in fish (Lane a = β – carotene authentic; Lane b = Astaxanthin authentic; Lane c = Carotenoid content of commecrcial fish ; Lane d = Carotenoid content of fish fed with algae; Lane e = Carotenoid content of fish fed with value added feed

Correlation matrix was developed using different variables like biochemical parameters of feed, fish and the growth parameters of the control and experimental fish. It was observed that feed protein and carbohydrate content were highly significant to fish protein and carbohydrate content of at a significant level of 0.01% interestingly it was observed that the fish carotenoid content was also significantly correlated to feed protein value rather than carotenoid content of feed. High significant correlation (at a significant level of 0.01%) was also recorded between feed protein and feed carbohydrate values to that of BWG, SGR, PER and PPV, whereas feed carotenoid value was significantly correlated to FCR at a significance level of 0.01% (Table 2).

Table 2. This represents the correlation matrix of the experimental fish and feeds after 30 days of rearing.

	Feed protein	Feed carbohydrate	Feed carotenoid	Fish protein	Fish carbohydrate	Fish carotenoid	BWG	SGR	FCR	PER	PPV
Feed protein	1										
Feed carbohydrate	.987**	1									
Feed carotenoid	-0.369	-0.372	1								
Fish protein	.906**	.899**	0.046	1							
Fish carbohydrate	.942**	.939**	-0.059	.993**	1						
Fish carotenoid	.890**	.883**	0.088	.998**	.986**	1					
BWG	.977**	.975**	-0.541	.814**	.869**	.789*	1				
SGR	.969**	.949**	-0.558	.793*	.845**	.768*	.992**	1			
FCR	0.332	0.332	-.997**	-0.089	0.015	-0.129	0.506	0.528	1		
PER	.850**	.864**	-.781*	0.572	0.652	0.539	.937**	.930**	.754*	1	
PPV	.879**	.876**	0.102	.997**	.985**	.998**	.780*	.757*	-0.144	0.528	1

** indicates significance at 0.01% level of significance

* indicates significance at 0.05% level of significance

BWG – Body weight gain%; SGR – Specific growth rate; FCR – Feed conversion ratio; PER – Protein efficiency ratio; PPV – Protein productive value.

The principal component analysis of biochemical parameters of the fish fed with 0, 50 and 100% replacement by algal biomass, after 30 days of experimentation was done (Plate 3). This analysis revealed that fish fed with control feed showed

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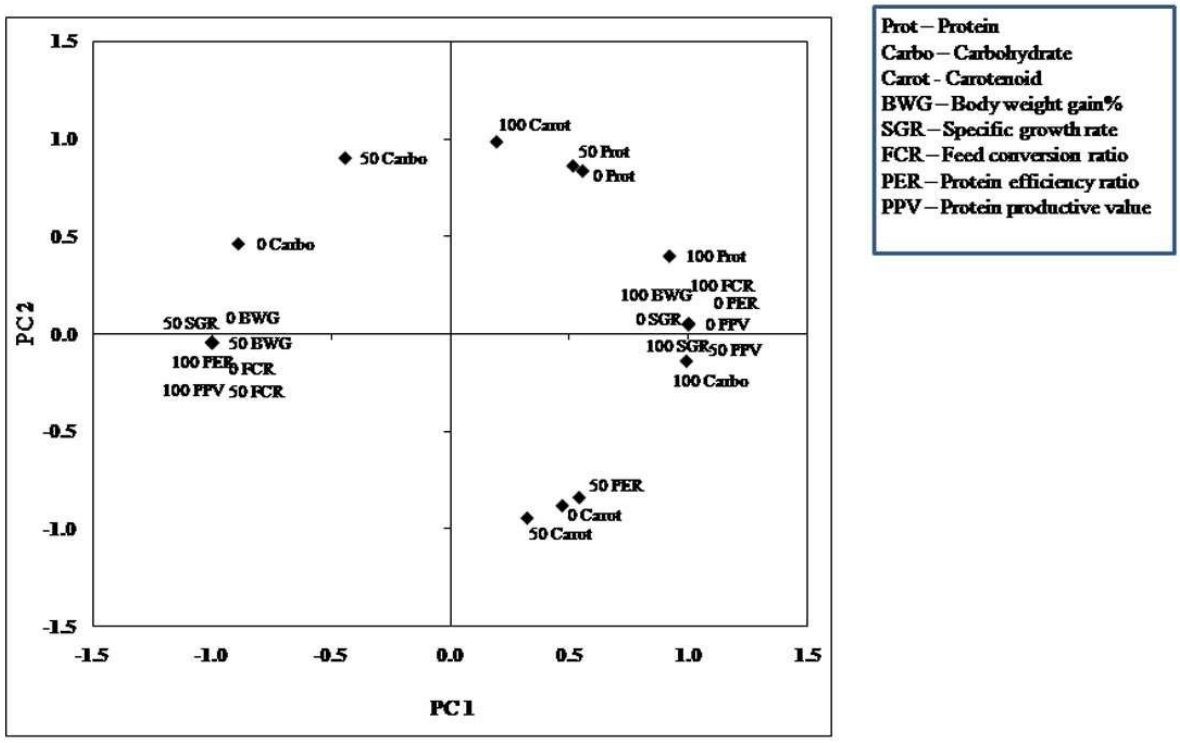
close relationship among protein content, PER, PPV and SGR (0 protein, 0 PER, 0 PPV, 0 SGR) and were positively correlated to both PC1 and PC2 (Right hand upper quadrate).

Plate 3. Photographs showing the Principal component analysis (PCA) of the biochemical parameters of experimental fish.

0%– Commercial feed fed fish .
50% - Value added feed fed fish.
100% - Algal feed fed fish .



Similar results were shown by Algae fed fish (100% replacement) for protein , FCR and BWG values (100 protein , 100 FCR , and 100 BWG) whereas carbohydrate content (100 carbohydrate) was closely related to SGR (100 SGR) and both of them are positively correlated to PC1 and negatively correlated to PC2. In the same way carotenoid value of the VAF fed fish and PPV and PER (50 PPV and 50 PER) were positively correlated to PC1 and negatively correlated to PC 2 (Right hand lower quadrate) indicating their relationships. Both PER and PPV of Algal feed fed fish are negatively correlated to PC1 and PC2.

Similarly BWG and FCR (50 BWG and 50 FCR) of VAF fed fish are negatively correlated to both PC1 and PC2 (Left hand lower quadrate) and from left upper quadrate of the diagram, it becomes evident that both carbohydrate and SGR value of VAF fed fish (50 carbohydrate and 50 SGR) were negatively correlated to PC1 and positively correlated to PC2, likewise carbohydrate content of control fish (0 carbohydrate) and BWG (0 BWG) showed the same results .Most significant change was noticed in protein content of VAF fed fish (50 protein) as it evident from PCA plot also showing its position in 1st quadrate.

Discussion

In aquaculture programs micro algal feed plays a very important role in promoting the skin coloration as well as the growth of the fish. Several studies have shown through years that micro as well as macro algae can act as a better feed supplement for fish (Pullin1987; Sommer et al .1990; Mustafa and Nakagawa 1995). Studies have also revealed a high value of dried microalgae as feed ingredient for crustaceans and fish larvae (Biendenbach et al. 1990; Navarro and Sarasquete 1998; Yuan and Chen 2000; Bar et al. 1995).

Skin coloration is highly important factor especially for ornamental fish. In the present study *Phormidium valderianum* has been found to promote the skin pigmentation of *Hemigrammus caudovittatus* in comparison to market feed. This is in full

agreement with the result observed in earlier studies of the present group (Khatoon et al. 2009), which showed the increase of carotenoid content in prawn and goldfish when fed with algae based feed containing *Nostoc ellipsosporum* and *Navicula minima*. The results indicated that microalgae like *Chlorella* had acted as a potential colorant in rainbow trout, whereas *Haematococcus* acted as a major β-carotene source for several aqua cultural programs (Choubert and Heinrict 1993; Gomes et al.2002).

Better health of the fish along with the color is always preferable in ornamental fish market. The above results have showed that VAF promoted better growth performance in fish along with increase in protein and carbohydrate content of the above. It was observed that there was a net increase in the BWG% as well as in the SGR% of the VAF fed fish. This result is in accordance with the observations of Khatoon et al. (2010a) which showed increase in body weight as well as specific growth rate percentage in gold fish on treatment with microalgae based feed. This result is also in agreement with those obtained by Dawah et al. (2002b) and Nandeesha et al. (1998) who found improvement in growth performance of Oreochromis niloticus on feeding with macro bacterial genus *Spirulina.* Similar result was also obtained by Badaway et al.(2008) on partial replacement of fish meal with *Chlorella* and *Scenedesmus* for Nile tilapia.

The mortality rate of the fish was also found to be much lower in case of value added fed fish as compared to others in the present study due to increased amount of carotenoids.The conversion of β-carotene obtained from algae to astaxanthin not only enhanced the color of the skin, but also acted as a potent antioxidant which helped to improve the immunity of the fish as observed in Tetras and Cichlids (Deventor and Heckman1996).This is also in full agreement with the observation of Gouroy et al.(2007), showing the mortality rate to be less than 10% in Nile Tilapia when fed with *Ulva* and *Cystoseira*. Since *Phormidium valderianum* has high carotene content, it helped the fish to survive better than the rest having some antimicrobial and antibacterial properties also. This has also been shown by the works of Manilal et al. (2009), which reputed a rhodophycean member *Asparagopsis taxiformis* to act as a potent antimicrobial agent in shrimps.

The correlation matrix studies revealed significant positive correlation between carotenoid content of fish and the protein and carbohydrate value. It means the protein component of cyanobacterial biomass served for both increasing protein content and the coloration of the fish as well.

Conclusion

Thus overall it can be concluded that *Phormidium valderianum*, due to its high nutritional value and pigment content having antioxidant property, can easily be used as feed ingredient for ornamental fish to achieve better results. This would not only act as pigment elicitor but also increase its immunity, thereby providing protection against bacterial and fungal diseases. Thus this would help to increase the market value of the ornamental fish in near future. The low cost biomass of this particular cyanobacterial genus may also replace other high value microalgae as fish feed ingredients.

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