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Effect of Formulated Algal Diet on growth performance of *Labeo rohita* Hamilton

Sudeshna Mukherjee[2], Dipannita Parial[1], Nilofer Khatoon[1], Atreyee Chaudhuri[2], Sudeshna Senroy[1], Sumit Homechaudhuri[2] and Ruma Pal[1*]
[1] Phycology Laboratory, Department of Botany, University of Calcutta
35 Ballygunge Circular Road, Kolkata – 700019, India
[2]Aquatic Bioresource Research Laboratory, Department of Zoology, University of Calcutta
35 Ballygunge Circular Road, Kolkata – 700019, India
* Corresponding author; Telephone no:+91-9433116320; E- mail: rpalcu@rediffmail.com

Abstract

A 12-week laboratory feeding trial was performed to evaluate the efficacy of two different algae based value added feeds (one containing *Spirulina platensis* and *Enteromorpha intestinalis* and the other with *Phormidium valderianum* and *Catenella repens*) against conventional (rice bran and mustard oil cake taken in 2:1 ratio, used as control) for fingerlings of Indian major carp, Rohu (*Labeo rohita*, Cyprinidae). The feeds were formulated on the basis of crude protein requirement of Rohu. Proximate composition of the above mentioned algal genera and the formulated feeds was analyzed in respect to protein, carbohydrate, lipid, carotenoid and ash content. Evaluation of diets was carried out on the basis of feed intake rate, body weight gain percentage, feed conversion ratio, specific growth rate, metabolic growth rate, protein productive value, protein efficiency ratio, total muscle protein, lipid, carotenoid, ash content and accumulated muscle glycogen content. It was observed that the value added algal feed 2 was more suitable diet than the other two for Indian Major Carp *L. rohita* fingerling as evident from the growth performances. On the other hand, control diet resulted into high body lipid deposition which led to poor growth of the fishes.

Key words: *Spirulina*, Rohu, Carotenoid, FCR, Specific Growth rate

Introduction

Rohu, Labeo rohita Hamilton, 1822, is one of the most cultured indigenous fishes in Indian subcontinent. Proper nutrition of this cultured fish is most important for high survival and rapid growth. Although previously fish meal was considered as the most balanced and suitable feed for the IMC, but because of the high cost and uncertain availability of fishmeal, the farmers are now compelled to search for cheaper alternative protein sources of either animal or vegetable origin (Kaushik, 1990; Higgs *et al.*1995). The nutritive values of various plant products have been evaluated through previous experiments so as to incorporate them in fish feed (Jackson *et al.* 1982; Ritcher *et al*. 2003; Adewolu 2008). Ray and Das (1994) studied the apparent digestibility of some aquatic macrophytes in *L. rohita* fingerlings. Ramachandran and Ray (2004, 2007) evaluated the nutritional value of extruded grass pea (*Lathyrus sativus*) seed meal and fermented black gram (*Phaseolus mungo*) seed meal in compound diets for *L. rohita* fingerlings. The influence of dietary Phytic acid on growth, feed intake and nutrient utilization in Juvenile Japanese Flounder (*Paralichthys olivaceus*, Paralichthyidae) was analyzed by Laining *et al.* (2010).

Interestingly algae have been proved to be one of the most important food sources and feed additives in the commercial rearing of many aquatic animals, especially fishes and penaeid prawn larvae (Borowitzka, 1997; Belay *et al.* 1996; Khatoon *et al.* 2010a, 2010b). A number of studies have assessed the nutritive value of dried microalgae as feed ingredients for crustacean and fish larvae (Biedenbach *et al.* 1990; Navarro and Sarasquete, 1998; Khatoon *et al*. 2009). Kumar *et al.* (2010) analysed the effects of periphyton and supplementary feeding with commercial pelleted feeds on the growth performance of juvenile Nile tilapia (*Oreochromis niloticus*, Cichlidae). Being a bottom and column feeder, Rohu prefers to feed on plant matter including decaying vegetation, unicellular and filamentous algae as reported by Jhingran and Pullin (1985). This lower group of plant contains a wide range of vitamins, pigments, nearly all essential nutrients including PUFA (Poly Unsaturated Fatty Acid) and also is a valuable source of proteins and carbohydrates. Because of the incredible impact of the algal genera on growth and vitality of fishes, a number of algae have been verified over time but less than forty genera have gained widespread use in aquaculture. Algal genera like *Skeletonema*, *Phaeodactylum*, *Chaetoceros*, *Pavlova*, *Isochrysis*, *Tetraselmis* are frequently used in aquaculture due to high content of PUFA, DHA (Docosahexanoic acid) and EPA (Eicosapentanoic acid) (Benemann, 1992; Becker, 1999; Wang, 2003; Knuckey *et al.* 2005). Other algal genera like *Spirulina*, *Chlorella*, *Scenedesmus*, *Dunaliella*, *Nannochloropsis* are widely used as aquaculture feed for their high nutritional value (Venkataraman, 1980; Avron and Ben-Amotz, 1992; Lee, 1997; Yamaguchi, 1997). *Spirulina*, *Dunaliella*, *Haematococcus* are also used as good sources of anti-oxidant pigments like carotenoids, lutein, astaxanthin, zeaxanthin, etc in fish farming mainly for colored fishes (Chiu *et al.* 2001; Hanaa *et al.* 2003) for the intracellular protection to fish larvae against different diseases together with the bright coloration of fishes (Trinadha *et al.* 2003).

Algae can be used directly as live culture or as value added feed supplement. Newly formulated algal diet should satisfy the nutritional requirements of the fishes with high acceptability of the feed. In the present study, the suitability of two algae based value added diets was evaluated against control feed (rice bran and mustard oil cake in 2:1 ratio) for promoting growth in Indian major carp *L. rohita* fingerlings over 12 weeks.

Material and Methods

Algal culture

Two cyanophycean algal genera, *Spirulina platensis* (Gomont) Geitler and *Phormidium valderianum* (Delp.) Gomont and two eukaryotic algae, *Enteromorpha intestinalis* (Linnaeus) Nees. of Ulvophyceae (Chlorophyta) and *Catenella repens* of Florideophyceae (Rhodophyta) were used as experimental materials. Among the algal genera used, *S. platensis* was mass propagated in vertical photobioreactor using Zarrouk's medium ($NaHCO_3$-13.61 g.l^{-1}, Na_2CO_3-4.03g.l^{-1}, K_2HPO_4-0.5 g.l^{-1}, $NaNO_3$-2.5 g l^{-1}, K_2SO_4-1 g.l^{-1}, NaCl-1 g.l^{-1}, $MgSO_4.7H_2O$-0.2 g.l^{-1}, $CaCl_2.2H_2O$-0.04 g.l^{-1}, $FeSO_4.7H_2O$-0.01 g.l^{-1}, EDTA-0.08 g.l^{-1}, Micronutrient solution-5 ml.l^{-1}) and *P.valderianum* was cultured in large tanks using Artificial Sea Nutrient medium (ASNIII containing – NaCl-25 g.l^{-1}, $MgCl_2.6H_2O$-2 g.l^{-1}, KCl-0.5 g.l^{-1}, $NaNO_3$ -0.75 g.l^{-1}, $K_2HPO_4.3H_2O$-0.02 g.l^{-1}, $MgSO_4.7H_2O$-3.5 g.l^{-1}, $CaCl_2$ – 0.5 g.l^{-1} , citric acid-0.003 g.l^{-1}, ferric ammonium citrate-0.003 g.l^{-1}, EDTA-0.0005 g.l^{-1}, Na_2CO_3-0.02 g.l^{-1}, trace metal-0.001l.l^{-1}) at 20˚C in 16/8 hour light/dark cycle under cool fluorescent light (intensity:- 20-30µ Einstein.m^{-2}.sec^{-1}). Natural collection of *E. intestinalis* and *C. repens* were used as other two algal resources.

Determination of proximate composition

Proximate composition of the above mentioned algal genera and the formulated feeds was analyzed in respect to protein, carbohydrate, lipid, carotenoid and ash content. Total protein, total lipid , total carbohydrate and total carotenoid were estimated following the standard assay methods of Lowry *et al.* (1951), Bligh and Dyer (1959), Hodge and Hofreiter (1962) and Sadasivam and Manickam (1996) respectively.

Formulation of feed

The rice bran and mustard oil cake in 2:1 ratio was considered as control feed. Two different algae based diets *viz.* Value added Feed 1 (VAF 1) (*S. platensis* and *E. Intestinalis* in 1.25: 1 ratio) and Value added Feed 2 (VAF 2) (*P. valderianum* and *C. repens* in 2: 1 ratio) were prepared using algal mix along with the control feed in proper ratio. Value added feeds were prepared on the basis of crude protein requirement of Rohu (40%) and the feed ingredient calculation was done following Pearson square formula (Mukhopadhyay, 1997).

Collection and rearing of experimental fishes

Fingerlings of *L. rohita* of same age group (body weight of 11.09 ± 0.49 g and total length of 10.77 ± 0.14 cm) were procured from local fish breeding farm near Kolkata, India and were brought to the laboratory in the oxygen packed plastic container. The fishes were acclimatized in the laboratory condition for 15 days in large glass aquaria (2' x 1' x 1') containing 40 l water. Stocking density was maintained at 12 fishes.40 l^{-1} water to avoid overcrowding and the fishes were treated with tetracycline bath (0.012g.l^{-1}) to prevent the outbreak of bacterial infection. Twigs of *Vallisneria spiralis*, *Pistia stratiotis* and *Jussiaea repens* were arranged properly in the aquaria to present a natural condition. The fishes were fed with live tubificid worm during acclimatization period and water was replenished daily to avoid accumulation of unutilized food and metabolic wastes. They were starved for 24 hrs prior to the onset of experiment.

Experimental procedure

The growth trial was carried out at a constant temperature of 25°C and controlled photoperiod (12D:12L) for a period of 12 weeks. In each of the nine aquarium, (2' x 1' x 1') containing 40 l filtered pond water 15 fishes were randomly allocated to make triplicate tanks for each feeding group (control and two VAFs). Supplemented aeration was provided to maintain dissolved oxygen near saturation. Other water quality variables such as ammonia, nitrite, and nitrate were maintained at acceptable levels by mechanical and biological filtration. The fishes were fed daily with their respective feed at 5% of body weight throughout the experimental tenure. The leftover food materials were collected daily and dried in incubator at 60°C for 24 hrs. The dried materials were weighed to measure feed intake. The water was replenished every day to avoid accumulation of unutilized food and metabolic wastes.

Initially and after successive intervals of three, six, nine and 12 weeks, body weight and total body length of every experimental fish were measured following Holčĭk *et al.* (1989). Six fishes were randomly selected from each experimental tank and decapitated to collect muscles for analyzing crude muscle protein, muscle glycogen, crude lipid and accumulated carotenoid content of the muscle.

Analysis of biochemical and growth parameters

Biochemical parameters such as total muscle protein, crude lipid content, accumulated muscle glycogen content and muscle carotenoid content were estimated following the same method as was done for estimating the proximate composition of algal genera and formulated feeds.

Various growth and nutrient utilization parameters of fishes *viz.* Feed intake rate (FIR), Body weight gain percentage (BWG%), Specific growth rate (SGR), Feed conversion ratio (FCR), Metabolic growth rate (MGR), Protein efficiency ratio (PER) and Protein productive value (PPV) were evaluated from each experimental feeding group as following Becker *et al.* (1999) and Siddhuraju and Becker (2003).

Statistical analyses

Univariate ANOVA following Zar (1999) was carried out at 5% level of significance to compare three feed means on the basis of morphometric and biochemical characters at selected periods of exposure during 12 week of continuous feeding.

Results

Determination of proximate composition of constituent algal genera and formulated feeds

The proximate composition of the selected algal genera and formulated feeds was determined and shown in Table 1 and Table 2 respectively. Among the four selected algal genera, *S. platensis* and *P. valderianum* showed proportionately higher protein content whereas, *E. intestinalis* and *C. repens* had high carbohydrate content in its nutrient composition. Those algae were formulated accordingly on the basis of crude protein requirement of *L. rohita* such that both the value added feeds would have similar proportion of nutrient content. The proximate composition analysis of the formulated VAF1 showed highest protein (48.61 ± 0.4%), carbohydrate (27.9 ± 0.4%), moisture (15.4%) and ash content (18.2%), whereas, VAF2 had higher muscle carotenoid (0.179 ± 0.1%) and gross energy (37.01%). The control feed had highest lipid content (10.11 ± 0.2%) among the three.

Table 1. Proximate composition of the algal genera used.

Algal genera	Protein (%)	Carbohydrate (%)	Lipid (%)	Carotenoid (%)
S. platensis	61.45 ± 0.4	5.7 ± 0.5	2.62 ± 0.4	0.08 ± 0.03
P.valderianum	25.62 ± 0.1	3.2 ± 0.3	3.19 ± 0.2	0.15 ± 0.34
E. intestinalis	13.05 ± 0.4	52.34 ± 0.3	4.58 ± 0.6	0.06 ± 0.02
C. repens	9.53 ± 0.3	32.24 ± 0.2	9.52 ± 0.3	0.18 ± 0.29

Table 2. Proximate composition of the formulated fish feeds.

Feed Type	Moisture (%)	Ash (%)	Protein (%)	Carbohydrate (%)	Lipid (%)	Carotenoid (%)	Gross Energy (%)
Control feed	12.6	11.2	40.89 ± 0.2	14.9 ± 0.5	10.11 ± 0.2	-	34.33
Value added Feed 1	15.4	18.2	48.61 ± 0.4	27.9 ± 0.4	4.93 ± 0.4	0.074± 0.01	35.61
Value added Feed 2	12.2	15.1	41.22 ± 0.3	20.3 ± 0.2	7.02 ± 0.2	0.179 ± 0.1	37.01

Feed intake rate

In the present study, the fish group fed with two experimental diets showed a higher feed intake rate than that of the control feed during the first 5 weeks of experiment (Fig 1). In the later stage, however, all the feeds were almost equally ingested by the fishes. On an average, the VAF 2 was mostly accepted (0.61 ± 0.14 g $fish^{-1}$ day^{-1}) followed by VAF 1 (0.599 ± 0.11 g $fish^{-1}$ day^{-1}) and control feed (0.56 ± 0.11 g $fish^{-1}$ day^{-1}).

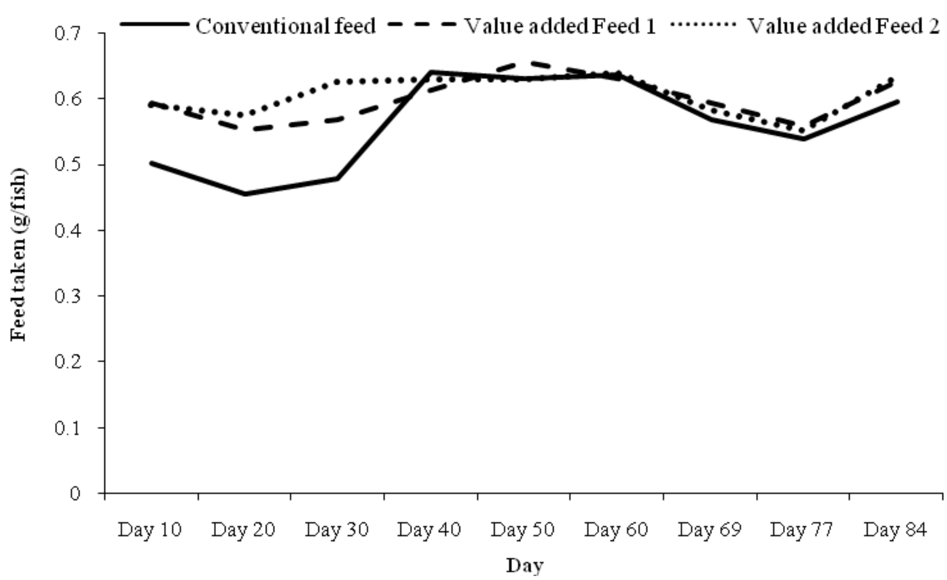

Figure 1: Feed intake rate of three groups of fishes fed with three different feeds.

Analysis of growth parameters

Inspite of almost equal feed intake rate, significant increases were found in the morphometric characters (body weight and total length) of the experimental fish groups as compared to that of the control group (P< 0.05). In course of the experimental tenure of 12 weeks, the fish group fed with VAF 2 showed almost 2.5 fold increases in body weight from an initial weight of 11.09 ± 0.27 g. VAF 1 also contributed a considerable increase accordingly. The total length of both the experimental fish groups increased from 11.9 ± 0.12 cm to 14.1 ± 0.15 cm for VAF1 and to 15.1 ± 0.16 cm for VAF 2. On the other hand, the fish group fed with control feed did not show any significant variation in the morphometric characters. Similar pattern of growth was reflected in various growth parameters studied. VAF 2 resulted in improvement of almost all the growth parameters (body weight gain percentage, specific growth rate, feed conversion ratio, metabolic growth rate) followed by VAF 1 and control feed (Table 3).

Table 3: Changes in different growth parameters of *L. rohita* fingerlings after continuous exposure of 12 weeks to two experimental diets as compared to control feed.

Growth parameters	Control feed	Value added Feed 1	Value added Feed 2
BWG %	25.068	126.691	144.725
SGR	0.266	0.974	1.065
FCR	1.6916	1.1258	1.02178
MGR	0.759	3.124	3.461
PER	0.147	0.432	0.550
PPV	-0.129	0.086	0.125

Analysis of Biochemical parameters

Univariate ANOVA showed that the muscle protein content of the fish group fed with VAF 2 increased significantly (P< 0.05) over 12 weeks of experimental tenure in comparison to the other two feeds (Table 4). The carcass protein of those fished rose to 21.35% from an initial 15.70%. Although, the VAF 1 had maximum amount of dietary protein, but the carcass composition of the respective experimental fish group showed only 2.5% increase in protein content over the tenure of 12 weeks. Negligible variation was found in the carcass protein content of the control group over the period. Almost same trend was followed when the analysis was done on the basis of accumulated muscle glycogen content. The fishes fed with VAF 1 could accumulate highest amount of muscle glycogen (26.5 ± 0.02%) followed by that with VAF 2 (21.3 ± 0.01%) and control group (18.6 ± 0.1%) within 12 weeks from an initial quantity of 12.1 ± 0.1%.

The muscle lipid content of the fish group fed with control feed increased significantly (P< 0.05) over the 12 weeks of experimental tenure in comparison to the other two experimental feeds, as confirmed by one way ANOVA (Table 4). The control diet resulted into a 2 fold increase in lipid deposition in the body. However, the other two experimental diets did not contribute any significant discrepancy in the muscle lipid content. In addition, it was also observed that the presence of carotenoid in the algal diets (VAF 1- 0.074% and VAF2- 0.179%) resulted into elevated deposition of the same in the algae fed fishes as compared to the control group (Table 4), the highest being for the fish group fed with VAF 2.

Table 4: Carcass proximate composition of *L. rohita* fingerlings fed on control and two experimental diets.

Feed	Week of Exposure	Body weight (g)	Total length (cm)	Protein (%)	Glycogen (%)	Lipid (%)	Carotenoid (%)	Ash (%)
Control	0	11.09 ± 0.27	11.9 ± 0.12	15.70 ± 0.03	12.1 ± 0.01	6.3 ± 0.02	0.08 ± 0.25	4.1 ± 0.27
	12	13.87 ± 0.13	11.97 ± 0.13	13.28 ± 0.02	18.06 ± 0.1	11.2 ± 0.01	0.07 ± 0.32	3.5 ± 0.31
Value added Feed 1	0	11.09 ± 0.27	11.9 ± 0.12	15.70 ± 0.03	12.1 ± 0.01	6.3 ± 0.02	0.08 ± 0.25	4.1 ± 0.27
	12	25.14 ±0.17	14.1 ± 0.15	18.51 ± 0.01	26.5 ± 0.02	4.41 ± 0.015	0.10 ± 0.12	4.7 ± 0.14
Value added Feed 2	0	11.09 ± 0.27	11.9 ± 0.12	15.70 ± 0.03	12.1 ± 0.01	6.3 ± 0.02	0.08 ± 0.25	4.1 ± 0.27
	12	27.14 ± 0.14	15.1 ± 0.16	21.31 ± 0.03	21.3 ± 0.02	4.43 ± 0.02	1.12 ± 0.21	4.4 ± 0.02

Discussion

Algae with high nutritional value have remarkable potential as fish feed. Previous finding by Khatoon *et al*. (2010b) suggested that algal feed could be a better supplement for animal protein like *Tubifex*, Tubificidae. Initially it was revealed from the proximate composition analysis of the formulated feeds as well as the carcass study that the value added feeds had higher ash content (VAF1-18.2% and VAF2-15.1%) than the control feed (11.2%) indicating more minerals in algal feeds, which resulted into high deposition of nutrients in the VAF fed fishes (Table 4), confirming that the experimental feeds enhanced more utilization of the dietary nutrients. The result was in contrast to the previous finding by Khatoon *et al*. *(*2010a) showing less utilization of the minerals in optimum level by more nutrient containing experimental algae based feed (*N. ellipsosporum*: *Navicula minima*: Daphnia:: 2:1:1) than the control one (Daphnia) as confirmed by ash content. This contrasting result might be due to the fact that the ability of fishes to utilize dietary nutrients may differ among species.

Several factors have also been reported to contribute to the observed variation in the growth responses of fishes. Hasan and Macintosh (1992) found that the growth performance of common carp (*Cyprinus carpio*, Cyprinidae_) varied with the variation in the acceptability of diets. This result was similar for the catfish (*Clarius batrachus*, Clariidae) as revealed by Hasan *et al*. (1989).

The fish group fed with two experimental diets showed a higher feed intake rate than that with the control feed during the experimental tenure. This might be due to the attractive colour, racy flavour and good nutrient composition of the experimental feeds. Previously Yamamoto *et al*. (2000) discovered that rainbow trout

(*Onchorhynchus mykiss*, Salmonidae) had a preference for diets with balanced essential amino acid pattern. Again Fournier *et al.* (2002) revealed that there was an effect of both protein and amino acid levels on voluntary feed intake in fish. Both VAF 1 and 2 had a good protein content for which the fishes seemed to prefer those foods.

In course of the experimental tenure of 12 weeks, the fish group fed with VAF 2 showed a substantial improvement in almost all the growth parameters (body weight gain percentage, specific growth rate, feed conversion ratio, metabolic growth rate) followed by VAF 1 and control feed (Table 3).The presence of a mixture of several algal proteins in proper ratio might have significantly enhanced growth as indicated by the previous findings. It was suggested that the use of various protein sources in combination was more effective than a single source in replacing fish meal in carp diet (Hasan, 1986; Hossain and Jauncey, 1990). Earlier reviews of Mustafa and Nakagawa (1995) confirmed that the supplementation of macro and micro algae meal enhanced growth, feed utilization, lipid metabolism, body composition, disease resistance and carcass quality of a variety of fishes. This was supported by the findings of Kumar *et al.* (2010) that the provision of bamboo substrate colonized with periphyton could significantly enhance the growth and yield of Nile tilapia grown in brackish groundwater ponds. The above finding hence pointed out that algal mixture might contribute to a good growth performance in long term exposure.

The high carcass protein content of the fishes from experimental feeding groups might have been contributed by the higher protein content of the experimental diets. Even though, the VAF 1 had maximum amount of dietary protein, it neither could convert into high carcass protein nor to the increased growth as was observed for VAF 2. Singh *et al.* (2006) revealed that specific growth rate, feed conversion and protein utilization of *L. rohita* fingerlings were influenced by the dietary protein content. The maximum growth of *L. rohita* fry resulted at a dietary protein level between 34.10 % and 36.60 % at a temperature range of 20 – 23º C (Akand *et al.* 1991) and the growth of tilapia *(Oreochromis mossambicus*, Cichlidae*)* showed a decrease in growth at dietary protein level above the optimum (Jauncey, 1982). The reciprocation of dietary proteins with growth and its utilization was also reported by Rajbanshi and Mumtazuddin (1989) for Indian major carp (*L. rohita*) when fed with different dietary protein levels. Hence, the VAF 2 having the optimum dietary protein level might be more suitable than VAF 1 to provide increased muscle protein and enhanced growth in fish body. Same trend was also observed in case of carbohydrate content as shown in Table 4. The high dietary carbohydrate content of the experimental feeds resulted into high muscle glycogen content.

The muscle lipid content of the fish group fed with control feed increased significantly ($P< 0.05$) over the 12 weeks of experimental tenure in comparison to the other two experimental feeds, as confirmed by one way ANOVA (Table 4). This might probably due to lipid deposition in the body of fishes fed with control feed containing high-lipid content. Deposition of high body lipid content in the fish fed on high-energy containing diet was also reported by Mahata (1994) and Nandeesha *et al*. (1990). The high lipid deposition in control diet group had a negative correlation with body weight gain which was supportive to the findings that the high energy content in the diets might have a negative correlation with weight gain (%) and specific growth rate (Page and Andrews, 1973; Daniels and Robinson, 1986). An inverse relationship between growth and dietary energy was reported by Daniels and Robinson (1986) in juvenile red drum (*Sciaenops ocellatus*, Sciaenidae). Our result corroborated with the previous findings that dietary lipid level above 15% could had an adverse effect on growth and food utilization as found in common carp (Bryant, 1980; Jauncey, 1979) and *Labeo rohita* (Hasan *et al.* 1993). On the other hand optimum lipid levels resulted in improved growth rates, feed conversion ratios, nutrient utilization, and reduced nitrogen excretion (Yigit *et al.* 2002; Martins *et al.* 2007). Both the VAF 1 and 2 had a lipid content of much lower than the optimum level and they resulted into a low body lipid deposition and an improved growth rate in all aspect. Therefore, the study confirmed the fact that the high lipid containing diet such as control feed used in this experiment might have a deleterious effect on fish body growth as excessive lipid could lead to decreased feed intake and might reduce the utilization of other nutrients, resulting in poor growth performance (Hemre and Sandnes, 1999).

The high dietary carotenoid content might have contributed to elevated muscle carotenoid deposition. The occurrence of carotenoid in the experimental fishes might protect them against different diseases as suggested by Trinadha Babu *et al.* (2003).

The overall study revealed that the VAF 2 contributed better growth performances as evident from most of the above parameters. On the other hand, control diet resulted into high body lipid deposition which led to poor growth of the fishes. The present experiment thus suggested that the efficacy of the algal protein incorporated diets was higher than the control one in terms of growth performances. Therefore, the locally available algal genera can be used in combination with the control feed to achieve a comparable or even better result in Indian major carp farming which may have a high commercial value in long term.

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