Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 7694 - 7704
Received 16 February 2021; Accepted 08 March 2021.

Enhancement of Growth and Colour of Pangasius Sutchi with Anabaena Variabilis as Fish Feed

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ABSTRACT

Fish form an important food resource for human beings. Both aquatic and marine fish have their own place in the human nutrition. Fish are also known for recreation as ornamental fish. The fish as part of human nutrition or as an ornamental delight require good nutrition for their growth. The marine fish are the major source of omega -3 PUFA required to be taken in diet (Saunders et al., 2013). These omega-3 PUFA are essential for healthy neuronal control and ageing (Swanson et al., 2012, Stark et al., 2016). The higher growth and better nutrition of the fish is thus required. By changing the environmental condition or by changing the mode of nutritional requirement of the fish the growth rate was tried to increase. But the cost for all these processes is very high and because of changing the physical and metabolic parameter, either toxic product is produced in the fish body or the quality of ornamental fish and the high nutritious quantity of the fishes used as food get decreased. We chose another way where the characteristics of the fish remain conserved without producing toxic product in a low-cost budget (Sarter et al., 2015; Chakraborty et al., 2015). Cyanobacteria like Anabaena variabilis which have the ability to scavenge nitrogen from the atmospheric dinitrogen gas often dissolved in water and the property of the antifungal, antibacterial, antiviral, immunosuppressed, antioxidant, and antioncogenic activity, can be used as a live food source for the fish (Goncalves et al, 2016) . Anabaena variabilis does not produce any toxic products and the physical characteristics like color, length, bodyweight of fish gave a positive result on being taken as food. The nutritional value of fish and the metabolic content like protein, oil, fat also got enriched while Anabaena variabilis was used as a food source.

Keywords:
Anabaena variabilis, PUFA, Antifungal, Antibacterial, Anti-Viral.

1. Introduction

Cyanobacteria traditionally known as biofertilizers are catching the imagination of the scientists with their varied uses. They are now being either used or being assessed for applications as varied as waste-water treatment, aquaculture feed, poultry feed, carbon sequestration and as a source for biofuel (Sood et.al. 2015, Goncalves et.al. 2016). Cyanobacteria with the relatively higher pigment content, are used as a source of colorants for food and cosmetic industries. Marine cyanobacteria such as *Lyngbya* and *Phormidium* have especially been studied for their antioxidant, anti-inflammatory and anti-cancerous activity, and production of secondary metabolites. These species are projected to be used for industrial production of medically important metabolites.

Cyanobacteria are the basis of the marine food chain being the major primary producers in the marine environment. In addition to the photosynthesis and nitrogen fixation, they have the unique ability of producing the polyunsaturated fatty acids. These PUFA especially the Omega-3 PUFA are then acquired by the marine fishes. The PUFA thus obtained from cyanobacteria is the major source of all the omega-3 PUFA derived from various cod liver oils. There is a major deficiency of n-3 LC-PUFA in diet, world over. Scientific groups are working towards finding solution to n-

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3 LC PUFA deficiency in diets and reduction in their quantity with respect to n-6 LC-PUFA.
The search for alternatives of fish oil based n3 LC PUFA has revealed two major candidates, plants and microbial oils. Seaweeds and microalgae have long attracted the attention of scientists and researchers as a source of various secondary metabolites and other resources such as n3 LC-PUFA (Silva et al 2013).
Scientists are also working towards increased lipid production in microalgae (He et al. 2016, Wang et al. 2016). Wang et al. (2016) have reported increase lipid production when stress was used in the form of suboptimal growth temperature together with chemical supplementation in the form of glycine betaine. The cyanobacterium *Synachocystis salina* was found to have enhanced lipid production in the presence of other cyanobacteria (*M.aeruginosa*) or green alga (*Pseudokirchaeriella subcapitata*) in co-cultures. (Goncalves et al. 2016)
Algae are the primary producers of n3 LC Fatty Acids. These microalgae grow both in the fresh water and marine water bodies. However, the type of algae in the two systems may differ in the n3 LCPUFA production. Chakraborty et al (2013) studied the composition and ratio of n3: n6 in fresh water mussel and found it to be lower than that in the marine species. This may be an indication of the ratio of these in the microalgae in the fresh water body. In a similar aspect the nutritional aspects of fish with respect to the amount of omega 3-fatty acids and other have been studied by Marichamy et al 2012. In a different study the processed fish in the form of flours was studied as a source of Fatty acid. The authors found a good quantity of c18 fatty acids together with various amino acids and minerals showing potential for their wide spread use (Vignesh and Srinivasan 2012).
Despite lot of research on omega acids, there is limited work on utilizing cyanobacteria as a source of n3 LC Fatty Acids, though they are the major primary producers for the fatty acids.
Most of the cyanobacteria are both nontoxic and nitrogen fixing. In addition, they may be producing secondary metabolites acting as deterrents of microbial pathogens. This led us to test the cyanobacterial samples as the fish feed. For this purpose, we chose the strain of *Anabaena variabilis* GITAM RGP isolated in our laboratory (hereafter referred to as *Anabaena variabilis*).
Several fishes such as American-flag fish, Siamese algae eater, and catfish of south America (otocinclus and Plecostomus species), Chinese algae eater, bristlenosecatfish and some freshwater shrimp like Amano shrimp generally eat algae. Some of these species of algae eaters feed exclusively on specific algae whereas others are able to feed on several types of algae. Most of the commercial fishes do not take live algal cells as their diet. Probably this is because they are reared on dry pellets, which may contain powder of dead Spirulina cells (Mukherjee et al, 2013).
Under normal environmental conditions, cyanobacteria and microalgae coexist with aquatic animals and fish without a problem. They are in fact the primary producers and necessary for the ecosystem. However too much growth caused by eutrophication, may lead to reduction in oxygen level of water. On the other hand, particular species of cyanobacteria and algae produce toxins which can cause toxicity, allergy or other discomfort to animal and even human beings exposed to the toxins. In aquaria the cyanobacteria may cause reduction in aesthetic values as they hide the fish from view. Several blue-green algae may spread rapidly over everything in the aquarium in slimy sheets, smothering and even killing off plant life. This the reason the aquaria need to be kept clean of unsightly algal levels by using algae-eating fish and manually cleaning the tank. Algae-eating fish have little effect on cyanobacteria, however, since these appears as a result of poor water quality. The cyanobacteria are normally controlled by water change and washing of the tank.
Cyanobacteria show a more diverse array of antioxidant compounds compared to most terrestrial plants. Phycobilin pigments, flavonoid s, Carotenoids, Catechin, glycosides, phlorotannins,

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sulphated polysaccharides, vitamins, phlorotannins and phenolic compounds are some major classes of antioxidant compounds reported from a variety of cyanobacteria and also some algae.
Anabaena variabilis GITAM RGP is a heterotrophic, photosynthetic, filam0entous cyanobacteria, having feature of both Gram negative and Gram-positive bacteria, as it contains an outer membrane with lipopolysaccharides and also contain a thick, highly cross-linked peptidoglycan layer (Stewart et al 2006).Various species of *Anabaena* sp. synthesize a variety of primary and secondary metabolites, many of them exhibit antifungal, antibacterial, antiviral, immunosuppressed, antioxidant and antioncogenic activity (Abdel- Raouf et al 2011). Few species of *Anabaena* are used as biofertilizer for rice crops to increases nitrogen content. Phytoremediation is another important application of *Anabaena variabilis* GITAM RGP which is responsible for removal of heavy metals from the polluted water bodies and industrial effluents (Pant et al 2012)

Ornamental Fish:
Fish keeping in captivity is an age-old practice. Chinese used a variety of containers for the purpose such as dishes, bowls and small tanks that permitted viewing from the top.
An aquarium unlike large water bodies is highly unstable with regard to environment for fishes housed in it. Water it holds is subject to very variable conditions which bring about rapid changes. Fish keeping in such water requires a good deal of careful management which is perhaps more important. An aquarium must be so set as to simulate the natural surroundings of fish as far as possible. It is therefore, to give natural touch to aquarium, the equipment's to regulate the pH, oxygen, temp., water quality is required. Ornamental fishes and aquatic plants are the main components of an aquarium. Some of the common Aquarium fishes are Gold fish of all varieties. Angle fish, Fighter fish, Gourami of all varieties, Guppy, Platy, Molly, Tangerine, Barbas, Sword tail etc. *Pangasius sutchi* is a variety of fish having a long body, latterly flattened with no scales Phu and Hein, 2003,Trong et al 2002). Head of this fish is relatively small, vornerine and palatal bones are present. Mouth of the fish is broad with small sharp teeth on jaw (Van de Braak, 2007 ; Gustiano ,2003). Eyes are relatively large and Two pairs of barbells are present among them upper shorter than the lower. The colour of this fish is mainly dark grey or black and six brached dorsal-fin rays are present . Gill rakers are normally developed in this fish eventually. Young fishes of *Pangasius sutchi* have black strip along lateral line and another long black stripe below lateral line (Van de Braak, 2007 ; Gustiano ,2003). Large adults are uniformly grey but sometimes it remains as greenish tint and silvery sides. Dark strips on middle of anal fin and dark strips in each caudal lobe are observed. Small grill rakers regularly interspersed with larger ones. It is an omnivorous i.e., it can take all type of food for fish. For natural growth of this fish, it needs a temperature of 22-28°c and pH of 6.0-7.5. under this optimum condition fish can grow significantly in the aquarium up to 30 cm. This fish looks a lot like shark and for suitable growth it needs a larger tank. *Pangasius sutchi* has never bred in aquariums.

Aim:
The present study was an attempt to observe the effect of using *Anabaena variabilis* GITAM RGP as a food source of *pangasius sutchi* and determine the changes of growth and compare the important factors like dry weight, protein content, amount of oil present, change of color etc. with using dry food as the food source of the fish.

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2. Materials And Methods

Blue Green 11(-) media was used for the cultivation of *Anabaena variabilis* GITAM RGP.

Components of the medium:

Component	gm/lL
K_2HPO_4	0.04 g
$MgSO_4 \cdot 7H_2O$	0.075 g
$CaCl_2 \cdot 2H_2O$	0.036 g
Citric acid	0.006 g
Ferric ammonium citrate	0.006 g
EDTA (disodium salt)	0.001 g
$NaCO_3$	0.02 g
Trace metal mix A5	1.0 ml
Agar (if needed)	10.0 g
Distilled water	1.0 L

The pH should be 7.1 after sterilization

Trace metal mix A5:

H_3BO_3	2.86 g
$MnCl_2 \cdot 4H_2O$	1.81 g
$ZnSO_4 \cdot 7H_2O$	0.222 g
$NaMoO_4 \cdot 2H_2O$	0.39 g
$CuSO_4 \cdot 5H_2O$	0.079 g
$Co(NO_3)_2 \cdot 6H_2O$	49.4 mg
Distilled water	1.0 L

Dry foods for fish:

Spirulina fish food is taken as a general food source for the fish. Which was fed to fishes two times per day at a specific time. The ingredients of this dry foods are –

1. Shrimp meal, cuttle fish meal, wheat flour, spirulina protease, thiamine, riboflavin supplement, soyabean meal.
2. Vitamin A, C, D_3, K, B_1, B_2, B_6, B_{12}
3. Mineral Zn,Co.Fe,Mn,Cu,P,Mg

Crude protein	**Crude fat**	**Crude fibre**	**Moisture**	**Crude ash**	**Nitrogen free extract**
Min 46%	Min 6%	Max 5%	Max 10%	Max 12%	20%

Fish:

24 *Pangasius sutchi* fishes were taken of almost same weight and almost same length and same color. All the fishes were having almost 6.0 cm in length and 1.02 gram of weight while they were taken.

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Pangasius sutchi

Aquarium:

Two big glass aquaria were taken which contained 3 chambers, each. Each of the chamber was same in height, weight and length. In each chamber 4 fishes can be placed for the experiment. Chamber size was 23cmx21cmx19cm (h x w x l). The aquaria were fitted with air pumps.

An aquarium containing 3 chambers with 4 fishes in each chamber

METHOD: From the 1st day of the experiment the food to fish were given into the tank in a equal ratio in each chamber twice a day i.e.at 10.00 am and 05.00 pm.

The ratio is-

Control (Dry foods) Chamber 1:	**Dry foods+ Algae Chamber 2:**	**Algae Chamber3:**
0.15 mg std fish food	0.075mg std fish food + 0.075 mg cyanobacteria	0.15 mg cyanobacteria

Here for each fish only 0.03 mg foods were given in each chamber. So, for 5 fishes total 0.15 mg foods are given once at a time.

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1. After every two weeks one fish was taken from each chamber and the dry weight of the fish, amount of protein, oil present in the fish, length of the fish was estimated and color of the fish was observed.
2. All these parameters were estimated at the beginning of this experiment by taking one fish from each tank to compare the further changes in the fishes after every two weeks of incubation and the data was noted down.
3. Step 1 and 2 was simultaneously carried out in the other aquarium at same time.

Weight measurement procedure:
To measure the weight of the fish first the fishes were taken out from the chamber then placed on the blotting paper for 30 minutes to soak the water. After that the weight was measured.

Length measurement:
The fish was taken from the chamber and then after measuring the dry weight it was placed on a white background to get the prominent colour of the fish. The length of the fish was measured using a thread and a centimeter scale.

Protein Measurement:
Protein was usually determined by measuring nitrogen, the characteristic element in protein, rather than protein itself. The nitrogen content of many proteins is about 16 per cent, and so the nitrogen content of a sample of fish was conventionally converted to crude protein by multiplying by 6·25. A common method used for food product (Sarvenaz and Sampels, 2017).
log whole fish N (g) = (log fish wet weight, g) x (slope) - (intercept).

calculations are only valid when the fish for which we were determining N content is within the same weight range as the group of fish originally used to derive the slope and intercept.
After calculating the amount of nitrogen present in the fish, Nitrogen values can be converted to crude protein using the formula:
crude protein = (N x 6.25).
now, from the table 1 we observed the slope of Pangasius sutchi is =1.09 &
the intercept of Pangasius sutchi is =1.77

Oil extraction & measurement:
The fish was cut and the head and the tail were removed completely. The rest of the fish can be cut into strips. The fish mass was made into a mush and the liquid was completely extracted with water. The resulting liquid was strained and kept in a water bath for heating till complete evaporation of water. The resulting oil content was measured in milliliters.


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Amount of oil extracted from the fish is measured

Fat measurement:

Fat was measured according to Modified Bligh & Dyer method. To 1 g of the prepared fish sample.

Added 3 ml methyl alcohol, 1.6 ml chloroform and enough water to make the total present in the jar up to 1.3 ml. The water added must include the water content of the sample. The ratio of water: methyl alcohol: chloroform was initially taken as 4:10:5. The mixture is blended for 2 minutes. Further extraction was completed as required with chloroform and water. Decant the filtrate from the Buchner flask to a 1 liter separating funnel, washing out the flask with a little chloroform. Allowed the filtrate to settle into an upper aqueous layer and a lower chloroform layer. The chloroform layer is collected and dried to a minimum volume on a rotary vacuum evaporator. This residual chloroform layer is transferred to a preweighed bottle, completely evaporated till the bottle shows constant weight. The weight gained is noted.

Fat content (%) = final weight of flask contents in grams × 2

By this method, more than 95 per cent of the fat content of the sample should be successfully extracted and measured.

With the help of the above procedures the parameters were obtained by taking out 1 fish from different test chambers of 2 aquarium after 2 weeks on specific day.

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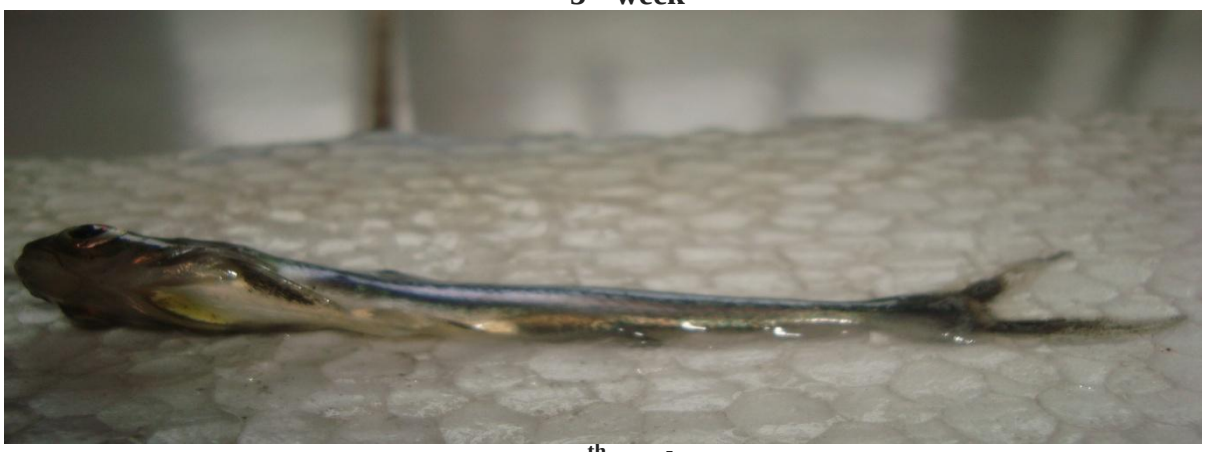
1st week

3rd week



7th week

Uptake of live algal cell as a food source by fish in 3rd chamber

3. Results and Discussion

In the current study *Anabaena variabilis* GITAM RGP was chosen as a food source for the selected ornamental fish named as *Pangusius sutchi*. Besides using only algae as a food source, normal dry food and mixture of both algae & normal dry food were used as a food source for comparative studies.

It was observed that the growth of fish which were using algae as a food was greater than other fish of the aquarium and colour of the fishes of mixture chamber and algal chamber was changed. But the growth rate of fishes of algal chamber was respectively greater than mixture and control

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chamber. Fishes of algal chamber are most glossy, dark greenish black in color where the fishes of mixture chamber were light greenish, brownish black in color and the fishes of control chamber were dark black in color.

The weight and length of the fishes of each chamber were measured after a specific time period and it was observed that the algal chamber fishes were having most weight and longest length after that the mixtures chamber fish and lastly the fishes of control chamber.

We can conclude that the growth rate of fishes of algal chamber is much greater and faster than the fishes of other chambers.

The protein, oil concentration and the fat percentage present in the fish body were measured and observed that algal chamber fishes were having most concentration of protein and oil rather than fishes of other chamber.

From this observation it was tabulated that fishes of algal chamber were healthier than the fishes of another chamber.

After measuring all the parameters, we can conclude that for a greater growth rate and maintain high concentration of nutritional value without producing any toxic product at a shorter time period, live algal cell of *Anabaena viriabilis* can be used as a best food source than the daily used dry food for the fish.

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Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 7694 - 7704
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Table 1: Results obtained from 1st chamber of two aquaria by taking average of both in which only dry foods was given as food are:

Batch of Fish, after 2 weeks	weight(gm)	Length(cm)	Protein content(gm)	Oil content (ml)	Fat content (%)	Color
1st batch (1st week)	1.0gm	4.5cm	0.625gm	0.7ml	20%	Fade black
2nd batch (3rd week)	1.29gm	4.9cm	0.625gm	0.73ml	20%	Medium black
3rd batch (5th week)	1.32gm	5.8cm	0.625gm	1.0ml	20%	Fade black
4th batch(7th week)	1.4gm	6.5cm	0.625gm	1.2ml	20%	Dark black

Table 2: Results obtained from 2nd chamber of two aquarium by taking average of both in which algae and dry food were given as food are:

Batch of Fish, after 2 weeks	weight(gm)	Length(cm)	Protein content(gm)	Oil content (ml)	Fat content (%)	Color
1st batch (1st week)	1.03gm	4.7cm	0.625gm	0.72ml	20%	Fade black
2nd fish (3rd week)	1.72gm	6.0cm	0.625gm	1.2ml	22%	Dark black
3rd fish (5th week)	2.11gm	6.9cm	0.642gm	1.6ml	25%	greenish black
4th fish (7th week)	2.23gm	8.0cm	0.642gm	1.65ml	25%	Dark greenish black

Table 3: Results obtained from 3rd chamber of two aquarium by taking average of both in which only algae was given as food are:

Batches of Fish after 2 weeks	weight(gm)	Length(cm)	Protein content(gm)	Oil content (ml)	Fat content (%)	Color
1st batch (1st week)	1.02gm	4.9cm	0.625gm	0.70ml	20%	Fade black
2nd batch (3rd week)	1.92gm	6.9cm	0.635gm	1.25ml	22%	Dark blue
3rd batch (5th week)	2.19gm	7.5cm	0.655gm	1.6ml	25%	Greenish blue
4th batch (7th week)	2.99gm	8.3cm	0.695gm	2.0ml	25%	Dark greenish blue