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Evaluation of Three Substrate Bound Periphyton Systems for the Farming of *Oreochromis niloticus* in South India

[1]K. Ranjeet and [2]P.V.P. Shahul Hameed
[1]Department of Fishery Hydrography, Kerala University of Fisheries and Ocean Studies, Panangad, P.O., Kochi-682501, India
[2]Department of Aquaculture and Fishery Microbiology, MES Ponnani College, Ponnani South, P.O., Kerala, PIN-679 586, India

Corresponding Author: K. Ranjeet, Department of Fishery Hydrography, Kerala University of Fisheries and Ocean Studies, Panangad P.O., Kochi-682501, India Tel: 0091-484-2700964 Fax: 0091-484-2703782

ABSTRACT

A cost-effective fish culture technology for *Oreochromis niloticus* was developed, whilst growing periphyton on three different substrates. The main objective of the study was to provide additional nourishment to the fish through supplementary feeding of periphyton as well as to reduce the accumulation of nitrogenous compounds in the water column through effective recycling of organic waste. Likewise, the suitability of the substrates (bamboo poles, PVC pipes and nylon nets) in cement cisterns were evaluated and compared based on the production characteristics and water quality parameters. Results indicated that the newly developed PVC supported artificial fibre system had greater aggregation of periphyton and thereby these treatments (TP-4) recorded high survival rates (66.6%), mean weight of fish (53.9±2.02 g) and net production (1928.9 kg ha^{-1}) compared to other two treatments and control. Similarly, the Food Conversion Ratio (FCR) and Specific Growth Rate (SGR) too were undoubtedly better in TP-4 (2.9 and 1.56, respectively), signifying the importance of adequate levels of supplementary feeding in these treatments. The levels of nitrite and ammonia in treatments with substrate bound periphyton were within permissible level indicating that the system effectively recycled the nitrogenous waste to valuable protein. With red tilapia culture gaining popularity in India, this is first such study from Kerala, South India, signifying the scope for wider acceptability among fish farmers in the region.

Key words: *Oreochromis niloticus*, periphyton, substrate, production, Kerala

INTRODUCTION

Aquaculture is a rapidly growing food producing sector. Compared to capture fisheries, aquaculture production world over has increased in manifold. This was mainly possible by the development of seed production and rearing technology for many finfish and shell (FAO., 2004). The intensive development of the aquaculture industry has been accompanied by an increase in environmental impacts. The production process generates substantial amounts of polluted effluent, containing uneaten feed and waste (Read and Fernandes, 2003). The high levels of nutrients cause environmental deterioration of the receiving water bodies. In addition, the drained water may increase the occurrence of pathogenic microorganisms and introduce invading pathogen species (Thompson *et al.*, 2002). Fish use proteins for energy production to a large extent, unlike terrestrial animals that use mostly carbohydrates and lipids (Hepher, 1988). Fish protein requirement, therefore, is about two to three times higher than that of mammals. In water, NH_3 and NH_{4+} are in equilibrium depending on the pH and the temperature (Timmons *et al*., 2002) and sum of the two forms the Total Ammonium Nitrogen (TAN). Although, both NH_3 and NH_4^+ may be toxic to fish, unionized ammonia is the more toxic form (Walsh and Wright, 1995). In most cases, the acceptable level of unionized ammonia in aquaculture systems is only 0.025 mg N L^{-1} (Neori *et al*., 2004; Chen *et al*., 2006).

Periphyton-based aquaculture is a recent concept and eco-friendly approach in the pond aquaculture. Periphyton is a preferable natural food for herbivorous and omnivorous fish species especially for Indian major carps (Keshavanath *et al*., 2002) and Tilapias (Azim *et al*., 2003). Periphyton has been reported to have positive effect on production of the target species and improving water quality (Beveridge *et al*., 1998; Wahab *et al*., 1999; Azim *et al*., 2001). Periphyton-based aquaculture is considered as an alternative to fish production in conventional, substrate-free ponds. The principle of traditional periphyton-based aquaculture is to introduce artificial substrates (e.g. wooden poles or bamboo) to enhance food availability via periphyton development and to increase the production of fish (Ramesh *et al*., 1999; Azim *et al*., 2004; Milstein *et al*., 2008). The bacterial film forming on these substrates attract periphyton to colonize on them. These are fed by herbivore fishes, which usually are grazers or scrappers. Not only does this provide a supplementary source of food to these fish but also improves the water quality by removal of excess load of ammonia from the system. However, a major disadvantage of the system is that in fishes that are voracious grazers, such as tilapia, the rate of feeding is intense as a result the time taken for periphyton to recolonize is high. Hence a continuous supply of periphyton is lacking. Another issue is the type of periphyton that colonize. It is possible that a totally new group of periphyton may prop up after the succession, which may not be preferred by the fishes. Hence, developing a system that can ensure regular availability of a particular periphyton is need of the hour.

Red tilapia, *Oreochromis niloticus*, is a recent introduction in Indian waters and very little has been assessed on its growth and potential as aquaculture species in Kerala. The present study is yet another step in developing a periphyton based aquaculture system with the help of probiotic bacterial association in the production system. The development of viable low cost technologies and their applications to current farming practices would help in enhancing aquaculture production. By providing suitable substrates, heterotrophic food production can be increased which will support the tilapia production and through usage of probiotics, there would be a higher aggregation of periphyton throughout the culture period irrespective of the nutrient load of the water column. Also it would improve the immunity of farmed species decreasing the occurrence of diseases. Under the above backdrop, a pioneering study was attempted to simulate substrate based periphyton production system in Kerala, South India, to assess the efficacy of this new method as a means for low budget farming of tilapia. The present system envisages an innovative system for periphyton based tilapia production which is environmental friendly and pivotal in popularizing substrate based sustainable aquaculture practice in the region.

MATERIALS AND METHODS

Experimental set up: The data for the present study were compiled from twelve preconditioned cement cisterns tanks of dimension (L×W×H) of 5×4×4 ft. at the fish rearing facility of MES Ponnani College during March-August, 2013. The average water holding capacity of each tank was maintained at 2000 L throughout the experimental period. Each tank bottom was filled with estuarine mud to a thickness of 10 cm. Lime was added at a rate of 500 kg ha^{-1} and water was filled to approximately half of the tanks capacity. At the end of first week, cow dung at a rate of 1000 kg ha^{-1} was added as phased manuring. Water was let in at the end of second week. Tanks were divided into four following completely randomized sampling method and additional substrates were erected in such tanks. At the occurrence of algal bloom, water was filled up to the desired level, which was maintained for rest of the experimental period. An air stone was provided in each tank and aerated during night hours. All the tanks were stocked with hatchery reared fry's of red tilapia *Oreochromis niloticus* (3.25±0.38 gm) procured from Aqua World Hatcheries, Thrissur and stocked at a rate of 50,000 ha^{-1}.

Four sets of combinations (treatments) were maintained for the study. Each set of treatment represented data collected from three separate tanks (triplicates). The first set of treatments (TP-1) included tanks in which no additional substrates for periphyton attachment was provided. In subsequent treatments, TP-2, TP-3 and TP-4, additional substrates in the form of bamboo poles, PVC pipes and artificial fibre wrapped PVC pipes were respectively used. The PVC pipes were of 4 inch diameter. For TP-4, the pipes were perforated and covered with a layer of non-degradable, non-corrosive artificial fibre of thickness 0.25 inch, throughout the structure, which was made intact by wrapping with discarded nylon net. The total additional surface area for all the three substrate-bound systems were rather same, providing 20% more area compared to that of the control (TP-1). The fishes were fed initially with high protein feed (Higashimaru scampi feed) equivalent to 10% of the fish biomass for one months and subsequently the feed ration was brought down to 5% of total body weight. Routine sampling of fish to ensure that they are devoid of any injuries or diseases was carried out on a fortnightly basis. At the end of each week, 0.5 g of probiotic Prebicom (Uni-Sankyo Ltd.), equivalent to 25 ms of *Lactobacillus acidophilus* and *L. sporogenes,* was mixed in one liter of water and transferred to the culture tanks.

Water exchange was minimal during the experimental period. The sides of the tanks were routinely scrapped for avoiding attachment of any periphyton. The scrapped mass was netted out. Throughout the period, water level lost either as evaporation or drainage was compensated and freshwater was added. Water quality parameters such as temperature, dissolved oxygen, transparency, water and soil pH were monitored on a monthly basis, following, AOAC (1990), while levels of Total Ammoniacal-Nitrogen (TAN) and nitrite-nitrogen in water samples collected during morning hours from each treatment were determined at fortnightly intervals using the Aquakit from Hanna. At the end of 6 months of culture periods, the tanks were dewatered by draining out and the fishes were harvested. All the fishes from each tank were examined on the day of harvest and were measured up to nearest millimeter and weighed up to nearest gram. The substrates were also scrapped per unit area and the periphyton per square inch were identified and counted in a haemocytometer. Based on the results, growth parameters, survival rate, production characteristics, Food Conversion Ratio (FCR) and Specific Growth Rate (SGR) for individual treatments were assessed and mean values of each treatment was compared using ANOVA.

Statistical analysis: The variations in the mean weight, survival rate and net production among the four treatments were tested employing Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984). Statistical analysis was performed using the Statistical Package for Social Science (SPSS 17.0 for windows, SPSS Inc., Chicago, IL, USA) (SPSS., 2008).

RESULTS

Water quality parameters among the four treatments did not show any significant difference ($p>0.05$) (Table 1) except for water pH, nitrite and ammonia, which varied significantly ($p<0.05$)

Table 1: Water quality parameters from the four treatments

	Treatments			
Parameters	TP-1	TP-2	TP-3	TP-4
Temperature (°C)	30.20±3.15	30.80±2.77	30.40±1.52	31.20±2.12
Dissolved oxygen (mg L^{-1})	40.38±1.62	50.26±0.84	40.82±0.91	50.52±1.02
pH range	80.80±0.46	70.70±0.52	70.40±0.64	70.50±0.55
Soil pH range	60.30±0.34	60.00±0.25	60.20±0.28	60.30±0.40
Transparency (cm)	24.50±12.40	32.50±18.60	35.00±11.30	28.70±13.80
Total alkalinity (mg L^{-1})	58.45±14.40	69.24±13.10	48.61±18.90	75.58±15.30
Nitrite-N (mg L^{-1})	40.11±0.05	00.14±0.03	00.12±0.04	00.11±0.03
Total ammoniacal N (mg L^{-1})	10.14±0.01	00.17±0.03	00.13±0.05	00.11±0.03

Values are means of four replicates (N = 72)

Table 2: Stocking details and yield characteristics of *Oreochromis niloticus* reared under different substrate levels

	Treatments			
Parameters	TP-1	TP-2	TP-3	TP-4
Stocking particulars				
No. per sq. ft	10	10	10	10
No. per ha	50.000	50.000	50.000	50.000
Mean weight (g)	3.2±0.32	3.2±0.22	3.2±0.38	3.3±0.38
Harvest details				
No. per sq. ft	3.66	5.33	6.33	6.66
No. per ha	19699	28687	34069	35845
Mean weight (g)	31.56±0.97	40.5±1.93	49.1±2.91	53.9±2.02
Net production (kg ha^{-1})	621.4±86.3	1161.5±125.8	1669.7±110.1	1929.8±100.4
Survival (%)	36.6	53.3	63.3	66.0

in treatment 1 (TP-1). In all other treatments the values were well within the permissible limits recommended for aquaculture. Table 1 shows the mean values for all the water quality parameters observed during the study period. Stocking and harvest details, such as mean weight of fish, percentage survival and net production from the treatments are summarized in Table 2. Final densities of *O. niloticus* at the time of harvest in treatments TP-1-4 were in order of 1.96±0.21, 2.86±0.25, 3.41±0.40 and 3.58±0.46 nos m^{-2}, respectively. An interesting observation of the study was that the retrieval rate from the tanks without any substrate for attachment of periphytons was significant lower than those provided with one or the other form of surfaces. This indicates that provision of artificial surfaces for attachment of periphyton has improved the survival rate from these tanks. Highest survival rate was recorded from TP-4 (66.6%), as compared to 36.6% registered from TP-1. Mean wet weight of fishes in the final harvested population also followed a trend similar to that of survival rate. Highest mean weight were registered in TP-4 (53.9±2.02 g), while least values in were recorded from TP-1 (31.56±0.97 g). The reduction in survival rate could also be directly attributed to increased levels of mean water pH, Nitrite and Ammonia concentration evidenced in TP-1. Since all the tanks were randomly selected and maintained in triplicates, chances for any reason other than non availability of periphyton may not be justified. Periphyton have been known to maintain good water quality conditions by converting toxic nitrite and ammonia to useful protein and thereby improving pH. The net production also varied considerably within the treatments. The least among the mean net production from treatments were recorded from those without any substrates (621.4 kg ha^{-1}), while the maximum production (1929.8 kg ha^{-1}) was recorded in treatments that had PVC and artificial fibre based substrates (TP-4).

Results of one-way ANOVA on means of the variables viz., Survival, Mean weight of fish and Net production are shown in Table 3. A noticeable difference for these variables was seen between

Table 3: Comparison of means for mean weight of fish, survival rate and net production among the four treatments

Parameters	Sum of squares	df	Mean square	F
Survival				
Between group	16.333	3	5.444	16.33**
Within groups	2.667	8	0.333	
Total	19.000	11		
Mean weight				
Between group	871.920	3	290.640	67.46**
Within group	34.467	8	4.308	
Total	906.387	11		
Net production				
Between group	3014183.543	3	1004727.848	88.30**
Within group	91023.547	8	11377.943	
Total	3105207.089	11		

**: p<0.01

Table 4: DMRT results for mean weight, survival rate and net production among the four treatments

	Mean weight		Survival		Net production	
Treatments	Mean	SD	Mean	SD	Mean	SD
TP-1	3.67[a]	0.57	31.57[a]	0.97	621.43[a]	86.30
TP-2	5.33[b]	0.58	40.50[b]	1.93	1161.53[b]	125.86
TP-3	6.33[b,c]	0.58	49.10[c]	2.91	1669.73[c]	110.17
TP-4	6.67[c]	0.58	53.90[d]	2.02	1929.87[d]	100.43

Means with same letter as superscript are homogeneous, SD: Standard deviation

the four treatments. On further analysis following Duncun's Multiple Regression Test (DMRT) it was evident that the difference followed a particular pattern, whereby for Survival and Net production all the four treatments were segregated into separate groups (Table 4). This further indicated that these parameters differed significantly (p<0.01) within the treatment as a result, the production characteristics for each treatment was perceptibly different. However, for mean weight, except for treatment 1 all other groups were closely associated. Although, performance of TP-4 was comparatively, different from TP-2 and TP-3, the differences with regard to mean weight were rather slender.

Variations in the Food Conversion Ratio (FCR) and Specific Growth Rate (SGR), variables identified for assessing the pattern of growth among fishes from the four treatment is illustrated in Fig. 1 and 2, respectively. Higher FCR, which indicate utilization of larger quantity of feed to attain unit weight was seen in TP-1 (4.9). On total contrast better FCR values were witnessed in treatments 3 and 4 (3.2 and 2.9, respectively). Results indicate that supplementary feeding of periphyton in both these treatments had immensely contributed to the increase in mean body weight. Since, the FCR values were nearly double between TP-4 and TP-1, it could be seen that the protein assimilation was high in fishes that fed on periphyton. In spite of greater protein utilization, the ammonia levels in these tanks remained with normal levels indicating that periphytons continuously converted excess nitrite and ammonia in the water to valuable protein. While, analyzing the variations in the Specific Growth Rate (SGR) values, similar results could be seen. The SGR, which indicates the growth attained for a period of time was desirably high in treatment incorporating PVC based substrates and least among treatments without any substrates. Results of DMRT also confirmed these finding whereby presence of PVC pipes wrapped with fibre substrates were found adequate for the growth and proliferation of periphyton compared to bamboo substrates, which once grazed by tilapia took a reasonable time to re-establish. Tilapia due to its prolific feeding nature continuously converts feed to protein. Hence, availability of quality protein increases the chance for better growth rate.

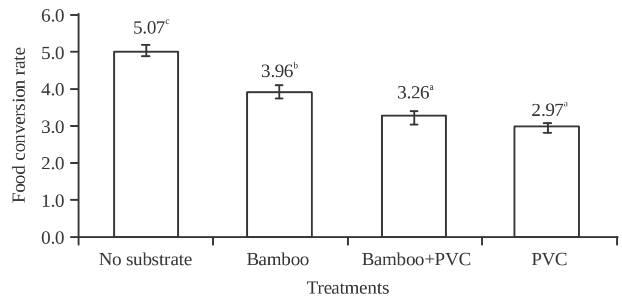

Fig. 1: Variation in the food conversion ratio of fishes in different treatments

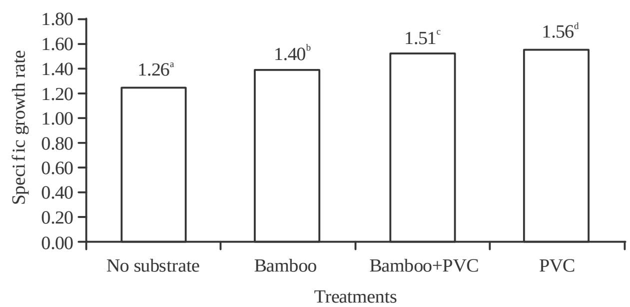

Fig. 2: Variation in the specific growth ratio of fishes reared under different treatments

While, analyzing the type of periphyton attached to each substrate and the number per unit area it was seen that there is a significant variation ($p<0.01$) in the periphyton assemblage. While the mean values from bamboo surface were 8.2 nos./sq.cm that recorded from PVC supported substrate was 18.7 nos./sq.cm. This difference could be attributed to two reasons. (1) Since, the PVC based substrate was more porous and thick compared to bamboo surfaces there was a greater tendency for the periphyton to get attached and (2) The biofilm which is the precursor for any periphyton colonization was multilayered as compared to single layered in bamboo. The multilayer biofilm formation was facilitated with the structural property of PVC pipes, which were hollow from within as well as perforated allowing water to seep into the pipe and allowing colonization of periphyton even at the basal layer. On closely analyzing the characteristic of periphyton present in different substrates it was seen that there is a wide diversity of periphyton biomass in the closed system. The periphyton identified were *Ulothrix* sp., *Pediastrum duplex*, *Chlorella* sp., *Selenastrum* sp., *Scenedesmus arcuatus*, *S. dimorphus*, *S. quadricauds*, *Closterium gracilae*, *C. lunula*, *Navicula pupula*, *Fragilaria intermedia*, *Mougeotia* sp., *Coelastrum* sp., *Lyngbya* sp., *Pleurosigma granulate*, *Staurastrum* sp. and *Melosira granulate*.

DISCUSSION

The natural food can increase the productivity and efficiency of aquaculture production systems (Azim and Little, 2006). Earlier studies have shown that in periphyton based aquaculture ponds, the addition of rigid surface to the oxygenated water column allows the development of attached autotrophic and heterotrophic population, which enhances nitrification and the development of algae autotrophic periphyton (Milstein *et al*., 2005; Van Dam *et al*., 2002). From a theoretical point of view, the biggest advantage of the added substrates is an increase in the energy and nutrient transfer efficiency of the system due to the additional periphyton-based production.

In periphyton-based systems, more nutrients are passed to higher trophic levels rather than accumulating in the system as in feed driven ponds. Nitrogen retention in higher trophic levels that can be harvested by the fish species is also higher in periphyton-based systems. Thus, the periphyton-based system offers the potential for increasing nutrient efficiency, reducing the accumulation of wastes in the system and improving the overall water quality. For an herbivore fish, such as *Oreochromis niloticus* feeding on small planktonic algae may not fully meet the energy requirements. Besides phytoplankton, these fishes generally require larger-sized food sources such as benthic algae, algal-based detritus or higher aquatic plants that can be harvested more efficiently (Asaduzzaman *et al*., 2009). Benthic algal mats rarely grow on bottoms in highly eutrophic environments due to light limitation. They need some hard substrate in euphotic layer of the rearing system to grow. Periphyton entraps organic detritus, removes nutrients from the water column and helps control the dissolved oxygen concentration and the pH of the surrounding water (Azim *et al*., 2002; Dodds, 2003; Bender *et al*., 2004). An ecological viewpoint, periphyton-based culture represent appropriate natural source of food for fish production (Hem and Avit, 1994) and performs better than the traditional substrate-free systems. The present study has made an attempt to utilize the water column for entrapping valuable protein that would otherwise be lost as detritus and thereby not only improving the production characteristics but also to maintain the water quality of the rearing environment.

The present work was envisaged with a view to develop a low-cost fish culture technology for red tilapia, *Oreochromis niloticus* as a pilot study in Kerala. The technology is based on increasing the production of natural fish food attached to surface areas (periphyton) by introducing artificial substrates in tank based aquaculture systems. The general objectives were to reduce the dependency on external resources and to increase nutrient and energy utilization, thereby improving simultaneously aquaculture sustainability and accessibility for poor farmers. The major objective was to ascertain the substrate suitable for periphyton synthesis and hence existing system of bamboo based periphyton culture was compared against that of newly developed PVC supported artificial fibre based periphyton production. The suitability of periphyton as food for fish, in terms of its increment in mean weight of the fish, survival rate and net production along with feed characteristics such as food conversion ratio and specific growth rate, was determined. In the present study production from PVC substrates were much higher than that of bamboo as reported by Asaduzzaman *et al*. (2009). Results indicate that supplementary feeding of periphyton in both these treatments had immensely contributed to the increase in mean body weight. Since, the FCR values were nearly double between TP-4 and TP-1, it could be seen that the protein assimilation was high in fishes that fed on periphyton. In spite of greater protein utilization, the ammonia levels in these tanks remained with normal levels indicating that periphytons continuously converted excess nitrite and ammonia in the water to valuable protein. Introduction of probiotics for enhancing the growth of periphyton in substrates is yet another highlight of the present study. Although, use of probiotics for increasing digestibility and better immune response has been studied by many researchers, very few reports have shown its use in developing periphyton. Through introduction of *L. acidophilus* and *L. sporogenes* in the water column, there occurred a continuous supply of biofilm producing beneficial bacterial around substrates, which manifested better growth of periphyton in the system.

Studies have shown that application of the periphyton treatment technique in the intensive aquaculture sector is not feasible. Nevertheless, the technique of using this natural feed may be significant, particularly in smaller, extensive-level aquaculture systems in developing countries.

In tropical countries like India and Bangladesh, mainly in the polyculture of Indian carps, with introduction of the substrates had a positive effect on consequent periphyton development, production of the target species and water quality (Beveridge *et al*., 1998; Wahab *et al*., 1999; Azim *et al*., 2002; Keshavanath *et al*., 2001, 2002). The results emerging from the present study fortifies this view, since there was a perceptible improvement in the production from tanks that incorporated periphyton. Earlier studies by Azim *et al*. (2001) had shown that bamboo as recommended as substrate for periphyton growth, in view of its production of high quality periphyton, its availability in the tropics, ease of use and durability. In the present study, a cost effective technology has been developed that provides better availability of periphyton. Since, periphyton attached on to the PVC based substrate are available for prolonged duration in spite of continuous grazing by tilapias, they can be considered better to bamboo poles. Periphyton substrates do not have any adverse effect on water quality parameters. By supplying a substrate area equal to the pond surface Azim *et al*. (2001) had stated that the periphyton alone could support a fish production of around 5,000 kg ha^{-1} $year^{-1}$. However, in the present study production from PVC substrates were much higher than that of bamboo. Hence, it can reasonably be concluded that fish production through incorporation of this technique could improve production beyond 5000 kg ha^{-1} $year^{-1}$. More research is needed to determine optimum substrate density and fertilization strategies and to select the fish species combinations that achieve the highest production. Other factors, such as the economic viability of the potential substrate materials will be important in determining how this technology can be applied under field conditions in large areas. Similarly, the FCR and SGR recorded in the present study was much better than that reported by Reboucas *et al*. (2012), that used a similar system for *O. niloticus*.

The present technology is based on increasing the production of natural fish food attached to surface areas (periphyton) by introducing artificial substrates in pond aquaculture systems. Results emerging from the study suggested that periphyton grown on PVC pipes and bamboo poles is an excellent natural food for certain fish species and supports enhanced fish production. However, while comparing the rate of production it was ascertained that PVC pipes wrapped with fibers performed better in enhancing mean weight, survival rate and net production among the different treatment. Fish production in the periphyton-based system using PVC support gave an increment of 82% for survival rate, 70.8% for mean weight and 210.6% for net production per ha compared to those without any provision of substrate. Since, these results were produced from small cement cisterns, under natural environment and in larger ponds, the results could be increased to manifolds. Hence, it could be fairly concluded that for any aquaculture production systems to be sustainable it is important to incorporate technologies that can be adopted at grass root level, which are both economically viable as well as environmental friendly. The results of the present study emphasizes the need to utilizing all ecological perspectives suitable for developing appropriate systems to reduce over dependence on commercial feed. In this regard, periphyton based production has a great role to play as evidenced in the present study.

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