

Evaluation of growth, survival and carcass composition of fish and prawn in the substrate based polyculture farming.

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Abstract

A series of substrate based aquaculture systems have been developed to reduce the cost of finfish and shellfish farming. Periphyton acts as important food component for finfish and shellfishes. The present study is aimed to assess the growth and survival of fish (*Labeo fimbriatus*, *Barbodes carnaticus*) and shell fish (*Macrobrachium rosenbergii*) in a substrate based system for a period of 6 months. Sugarcane bagasse is used as a substrate for the growth of periphytons. The experiment was carried out in three treatments, T₁ (only substrate), T₂ (substrate + feed) and T₀ (only feed/control) with triplicates. The results of the study showed maximum average weight in T₂ (*L. fimbriatus* 141.17 ± 0.66; *B. carnaticus* 98.07 ± 1.16 and *M. rosenbergii* 73.95 ± 0.73) compared with T₁ and T₀ and there is a significant difference (P<0.05) observed between the treatments, substrate showed significant effect the water quality parameters viz., pH (6.73-8.17), dissolved oxygen (6.70-8.50 mg/l). Low concentration of Ammonia (0.068-0.140 mg/l) and Nitrite (0.046-0.082 mg/l) was observed in substrate based ponds. The results of substrate based cultural system increased the protein content of the fish and prawn. The study revealed the relative advantage of using sugarcane bagasse in polyculture system for enhancement of health and growth of the fish with increasing the production.

Keywords: Fish and shellfish, Polyculture, Water quality, Sugarcane bagasse, Periphyton.

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Introduction

Over the years, the semi-intensive systems of fish farming are highly subjected to use of feed and fertilizers. However, these leads to increase in the cost of production and most of the time given food is not consumed by animal and finally goes to environment as fish waste when it is discharged into the water bodies causes problem of eutrophication [1,2]. Also, high feed costs are unbearable for small and marginal fish farmers. Therefore, from the economic and environmental points of view, there is a need to make semi-intensive aquaculture systems more nutrient-efficient.

In any aquatic system, autotrophic and heterotrophic are two important feeding pathways for fish, as they play major role in the nutrient cycling as well as carbon flow in water column. In most of the culture systems, two basic food sources present for all organisms viz., primary productivity from algae, where secondary trophic feeders such as zooplankton, benthos including fish utilizes organic matter produced by algae. Secondly, the organic matter added as feed or manures, to enhance productivity by direct or indirect consumption by fish. In both cases, heterotrophic microorganisms (bacteria, fungi, protozoa) are essentially important where they decompose organic matter and releases nutrients which either utilized by algae or taken by cultured organism [3]. One can reduce the cost of artificial feed via enhancing autotrophic and heterotrophic production in culture pond by raising fertilization rate. However, autotrophs are light dependent and promotion of heterotrophs in

the pond can be carried out during night time by using different substrate via periphyton technology [4].

Periphyton is considered as an important food component for finfishes and shellfishes. It is being used traditionally as rich aquatic feed for fishes throughout the countries like Cambodia, West Africa, Srilanka, India and Bangladesh. The principle of periphyton-based aquaculture is to provide substrates on which bacteria, protozoa, fungi, phytoplankton, zooplankton, benthic organisms, and a range of other invertebrates colonize and which acts as source of feed for fishes. This periphyton, traps dissolved and suspended nutrients from the water matrix and in turn, increases natural feed to cultured organisms. Periphyton is effectively utilized by many fish species which thrive low in the food chain [5, 6]. Along with food production, periphyton acts as water purifier by means of nutrient cycling and retention of nitrogenous compounds [7]. Besides, substrates are shelters for avoiding agonistic behavior in prawns, which ultimately increases yield [8].

Many researchers have demonstrated the use of different substrates such as bamboo for *Tor khudree*, *Labeo fimbriatus*, *Oreochromis niloticus* and *Macrobrachium rosenbergii*, plastic sheet and ceramic tile for brackishwater shrimp, rice straw mats for Nile tilapia and sugarcane bagasse for *Labeo rohita* and *Etroplus suratensis*, to improve water quality, enhance growth and increase production [9-14]. Sayed stated that periphyton can partially or totally replace or complement supplemental feed in phytophagous tilapia ponds, without reducing fish

yield but with considerable reduction in production cost. Thus, this aquaculture technique can be an excellent alternative to reduce production cost and allow an economically viable fish production, particularly in rural, resource limited regions in developing countries [15].

Furthermore, there is a great scope for enhancement of heterotrophic food production by using easily available agricultural wastes like sugarcane bagasse, paddy straw along with fertilizers/manures, these serves two important purposes viz., it can be converted to protein rich microbial biomass and at the same time reduces the problem of waste disposal[16]. Gangadhar and Keshavanath evaluated bagasse was good periphyton substrates in terms of periphyton ash free dry matter, chlorophyll-a and nutrient composition. The earlier studies were based on the viability of periphyton-based aquaculture technology and there is very less work on the impact of these substrates on composition of fish body composition. The present study was aimed to study the influence sugarcane bagasse on growth, survival and carcass composition of *Labeo fimbriatus*, *Barbodes carnaticus* and *Macrobrachium rosenbergii* in polyculture system.

Materials and Methods

Experimental design

The experiment was conducted for a period of 180 days at the Fisheries Research Information Centre (Inland), Hesaraghatta (13°8'18.8088"N and 77°28'40.4040" E), Bengaluru, and Karnataka, India from Nov-2019 to Apr-2020. The experiment was carried out in cement cisterns, each with size of 20 m² in open system. One control and two treatments of sugarcane bagasse substrates in triplicates were tried: No substrates, substrates and Substrate+feed. Here in called treatments T₀, T₁ and T₂ respectively.

Sugarcane bagasse was soaked in water for two days in order to remove excess sugar and then sun dried properly. The bagasse was made into bundles of approximately 7.5 cm diameter and 1 m length by using nylon rope [17]. Bagasse bundles were hang vertically at rate of 5000 kg/ha in T₁ and T₂ with the regular intervals by using steel wire, across the pond by maintaining uniform distance of 20 cm between bagasse and tank inner surface as a free border zone and thus a total 20 bundles were tied in each pond.

Pond preparation, fish stocking and feeding

The experimental ponds were dried initially for a week to eradicate aquatic weeds and animals. Soil base of 15 cm was added and lime was applied at a rate of 250 kg/ha. On the following day water was filled in all tanks, and bagasse was installed in ponds according to experimental design.

After a week of liming, all ponds were fertilized with semi-decomposed cow dung, urea and Single Super Phosphate (SSP) at a rate of 3000, 100, and 100 kg/ha respectively and thereafter manuring were continued fortnightly with the same dose till the end of experiment. The water level in the tanks was maintained at 90 cm ± 5 cm throughout the experimental period, and ponds were allowed for 7 days for the good growth of periphyton on bagasse bundles and planktons in water column.

Fingerlings of two indigenous fishes *L. fimbriatus* (35.97 ± 0.61 mm; 11.89 ± 0.67 g), *B. carnaticus* (13.39 ± 0.09 mm; 0.69 ± 0.02 g) and freshwater prawn larvae of *M. rosenbergii* (38.66 ± 0.88; 9.01 ± 0.28 g) were stocked at ratio of 3:3:4 respectively, with a total stocking density of 10,000/ha (i.e. 6, 6 and 8 individuals per pond respectively). Pelleted sinking feed of 30% protein were given to the treatment T₀ and T₂ at 5% of body weight in two weeks, there after it was reduced to 2% as per the body weight. The fishes and prawn were fed two times a day during the experimental period.

Water quality monitoring

Water samples were collected between 09.00 to 10.00 morning hours on weekly interval and analyzed for transparency (Secchi disc), temperature (mercury thermometer) and pH (pH meter) and nutrients concentration [total ammonia-N (TAN), nitrite-N (NO₂-N), nitrate-N (NO₃-N) and phosphate-phosphorus (PO₄-P)] by following standard protocols.

Growth performance and harvesting

Fishes and prawns were sampled fortnightly for regular assessment of growth and biomass. Substrates were removed before sampling and ponds were dragged to catch at least 50% of each species from the pond. The length and weight of each individual were recorded in each sampling. At the end of the experiment, fishes and prawns were harvested by draining the ponds. Species-wise SGR (% body weight/day), Survival (%), Gross yield (kg/ha) were calculated by following formulae:

$$SGR (\% / d) = \frac{\ln(W_{harv}) - \ln(W_{stoc})}{\text{No of Days}} \times 100$$

$$Survival \% = \frac{N_{harv}}{N_{stock}} \times 100$$

$$Total yeild (kg / ha) = \frac{W_{harv} \times N_{harv} \times 10000 \times 60}{1000}$$

Proximate carcass composition

The carcass was analyzed for fish and prawn for crude protein, lipid, ash and moisture using the methods of AOAC (2016) at the beginning and end of the study. For this, muscle tissue of fish and prawn from each treatment were used.

Statistical analysis

All statistical analyses were done using SPSS 20.0. One-way ANOVA followed by Duncan's multiple range test (P<0.05) was applied to find out the significance difference and homogeneity between the treatments.

Results

Water quality parameters

Physico-chemical characteristics of water recorded in the experiment are given in Table 1 and Figure 1. Except water temperature (20.03-29.07 °C), all other parameters like transparency (22.07-24.70 cm), pH (6.73-8.23), dissolved oxygen (6.07-8.53 mg/l) total alkalinity (120.00-215.67 mg/l) and hardness (73.33-108.33 mg/l), also nutrients such as NH₃-N (0.068-0.167 mg/l), NO₂-N (0.046-0.094 mg/l) NO₃-N (0.069-

Table 1. Water quality parameters.

Treatment	Temperature (°C)		Transparency (cm)		pH		DO (mg/l)		Tot. alkalinity (mg/l)		Tot. hardness (mg/l)		NH ₃ -N(mg/l)		NO ₂ -N(mg/l)		NO ₃ -N(mg/l)		PO ₄ -P(mg/l)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
T ₀	26.30	0.45	24.00	0.05	8.00	0.03	7.50	0.15	160.60	4.70	98.40	1.25	0.104	0.003	0.075	0.002	0.09	0.002	0.04	0.002
T ₁	25.60	0.49	23.50	0.06	7.70	0.07	7.50	0.09	177.40	5.93	87.20	0.61	0.089	0.003	0.063	0.002	0.093	0.001	0.036	0.002
T ₂	25.50	0.48	23.10	0.14	7.70	0.08	8.00	0.09	191.70	3.79	82.10	1.26	0.092	0.002	0.064	0.002	0.09	0.002	0.035	0.002

Note: The values of different water quality parameters are mean ± SE of three ponds per treatment.

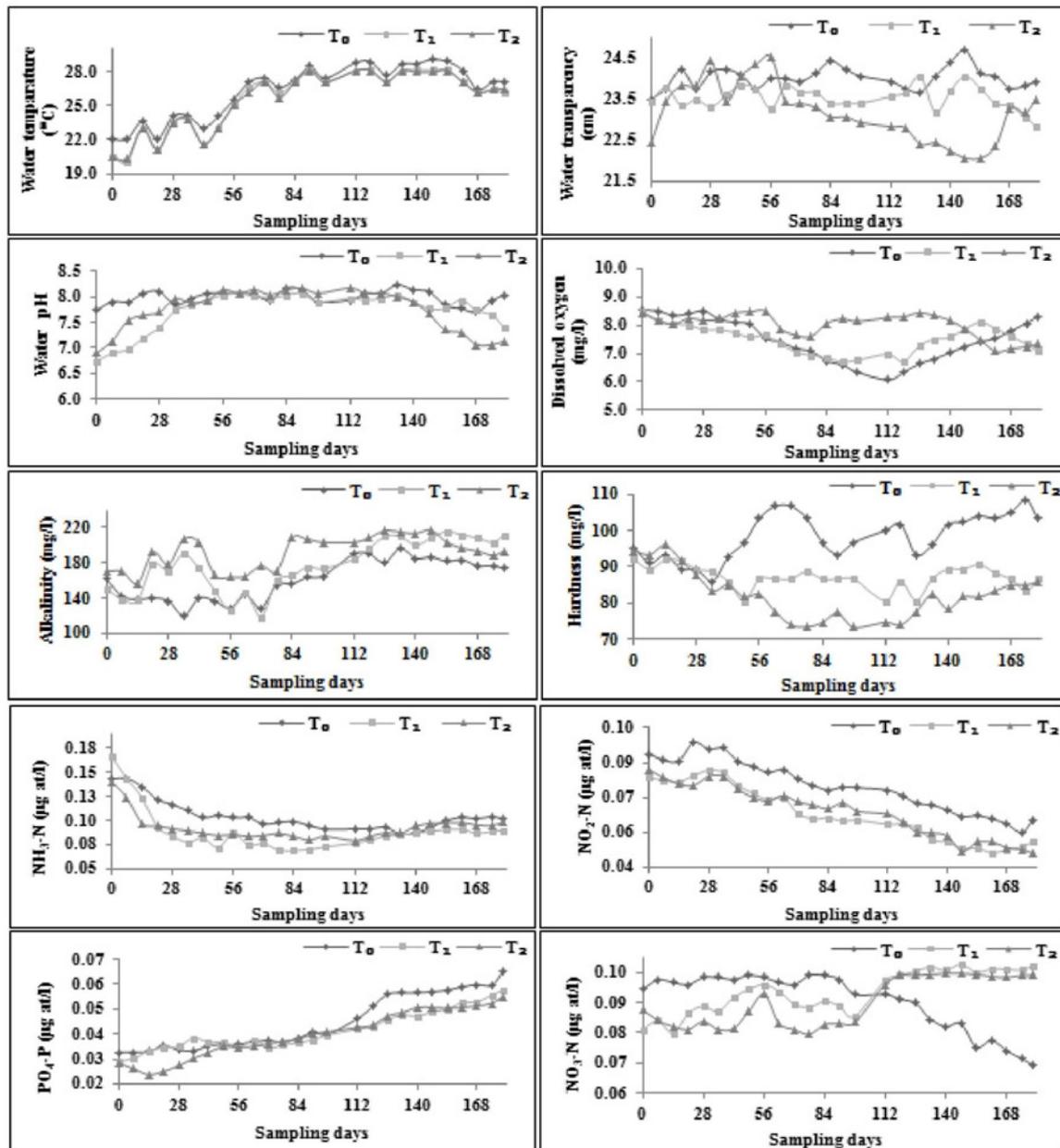


Figure 1. Water quality parameters.

0.99 mg/l) and PO₄-P (0.019-0.060 mg/l) showed significant difference between treatments and control ponds (P<0.05). DO concentration showed decreasing trend initially and the concentration was found high in T₁ and T₂ than T₀. Low water pH and transparency observed in substrate treatments (T₁ and T₂) than the control. Total alkalinity concentration was found high in substrate treatments compared to control, whereas reverse trend observed for total hardness. The nutrients concentration NH₃-N, NO₂-N and PO₄-P was found low in substrate, however

NO₃-N found high in substrate ponds.

Fish survival, growth and production

Labeo fimbriatus (141.17 g; 120.30 g), *Barbodes carnaticus* (98.07 g; 80.57 g) and *Macrobrachium rosenbergii* (73.95 g; 55.90 g) were showed good growth in T₁ and T₂ tanks respectively compared to T₀ group (132.20 g; 86.73 g; 54.47 g for respective species). The highest survival observed in T₂ (89.81%) compared to T₁ (85.19%) and T₀ (83.33%) and gross

Table 2. Fish and prawn growth parameters.

Treatment	Species	W _{stock} (g)		W _{harv} (g)		Survival (%)		SGR (%/d ⁻¹)		Gross yield (Kg ha ⁻¹)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
T ₀	Lf	10.67	0.90	132.20	1.54	83.33	9.62	1.39	0.04	988.95	34.62
	Bc	0.67	0.03	86.73	1.27	83.33	9.62	2.71	0.04	648.40	22.12
	Mr	8.33	0.26	54.47	2.08	83.33	4.17	1.04	0.03	545.90	14.24
T ₁	Lf	12.90	1.60	120.30	0.38	88.89	5.56	1.25	1.25	962.35	19.93
	Bc	0.67	0.03	80.57	0.83	83.33	9.62	2.67	0.03	605.35	25.05
	Mr	8.80	0.46	55.90	4.06	83.33	4.17	1.03	0.01	562.15	21.28
T ₂	Lf	11.90	0.95	141.17	0.66	94.44	5.56	1.38	0.05	1200.5	25.10
	Bc	0.73	0.03	98.07	1.16	88.89	5.56	2.72	0.03	784.60	16.78
	Mr	9.90	0.21	73.95	1.73	91.67	8.33	1.12	0.01	814.15	25.94

Note: The values of growth parameters are mean ± SE of three ponds per treatment (Lf: *L. fimbriatus*; Bc: *B. carnaticus*; Mr: *M. rosenbergii*).

Table 3. Analysis of variance (ANOVA) comparing the fish growth between treatments.

Species	Effect	df	MSS	F ratio	P value
<i>L. fimbriatus</i>	Between groups	2	1235.351	.727	0.485
	Within groups	114	1698.179		
	Total	116			
<i>B. carnaticus</i>	Between groups	2	1120.381	1.363	.260
	Within groups	114	822.044		
	Total	116			
<i>M. rosenbergii</i>	Between groups	2	1501.566	3.672	.028
	Within groups	114	408.908		
	Total	116			

Note: df: Degree of Freedom; MSS: Mean Sum of Squares.

Table 4. Carcass proximate composition of fish and prawn.

Treatment	Protein (%)	Fat (%)	Ash (%)	Moisture (%)
<i>L. fimbriatus</i>				
Initial	13.20 ± 0.01	3.69 ± 0.01	1.40 ± 0.10	78.33 ± 0.04
T ₀	16.29 ± 0.17	6.36 ± 0.10	1.82 ± 0.07	74.89 ± 0.33
T ₁	16.90 ± 0.06	5.29 ± 0.04	2.65 ± 0.09	74.36 ± 0.27
T ₂	16.97 ± 0.07	5.50 ± 0.40	2.40 ± 0.34	75.09 ± 0.16
<i>B. carnaticus</i>				
Initial	18.72 ± 0.02	2.88 ± 0.06	1.58 ± 0.03	73.36 ± 0.03
T ₀	20.63 ± 0.35	4.33 ± 0.09	2.29 ± 0.02	70.72 ± 0.29
T ₁	21.58 ± 0.04	4.13 ± 0.11	2.20 ± 0.03	70.52 ± 0.29
T ₂	21.79 ± 0.15	4.00 ± 0.01	2.05 ± 0.13	69.57 ± 0.36
<i>M. rosenbergii</i>				
Initial	16.82 ± 0.01	5.21 ± 0.02	2.12 ± 0.02	76.55 ± 0.03
T ₀	19.02 ± 0.13	8.21 ± 0.18	3.21 ± 0.07	70.36 ± 0.19
T ₁	19.89 ± 0.20	7.36 ± 0.27	3.12 ± 0.07	71.05 ± 0.65
T ₂	20.03 ± 0.13	7.64 ± 0.08	3.01 ± 0.06	69.18 ± 0.51

Note: The values of carcass composition are mean ± SE of three ponds per treatment.

production (Kg/ha) also showed high in T₂ (932.65) followed by T₀ (728.89) and T₁ (708.56). There is a significant difference (P<0.05) observed between the treatments. Fish and prawn growth, survival and production are given in Tables 2 and 3.

Carcass composition

The fishes and prawn showed high carcass protein content in T₂ compared to T₁ and T₀. The protein content for *L. fimbriatus* (16.97 ± 0.07), *B. carnaticus* (21.79 ± 0.15) and *M. rosenbergii* (20.03 ± 0.13) was observed in T₂ compared to T₁ (16.90 ± 0.06; 21.58 ± 0.04; 19.89 ± 0.20) and T₀ (16.29 ± 0.17; 20.63 ± 0.35; 19.02 ± 0.13) for respective species. Similarly, fat content (5.50 ± 0.40), (4.00 ± 0.01), (7.64 ± 0.08) and ash content (2.40 ± 0.34), (2.05 ± 0.13), (3.01 ± 0.06) for *L. fimbriatus*, *B. carnaticus* and *M. rosenbergii* respectively in T₂ compared to T₁ (5.29 ± 0.04; 4.13 ± 0.11; 7.36 ± 0.27), T₀ (6.36 ± 0.10; 4.33 ± 0.09; 8.21 ± 0.18) for fat and T₁ (2.65 ± 0.09; 2.20 ± 0.03; 3.12 ±

0.07), T₀ (1.82 ± 0.07; 2.29 ± 0.02; 3.21 ± 0.07) for ash content. Moisture content in fish and prawn found low in T₂ (75.09 ± 0.16), (69.57 ± 0.36), (69.18 ± 0.51) compared to T₁ (74.36 ± 0.27; 70.52 ± 0.29; 71.05 ± 0.65) and T₀ (74.89 ± 0.33; 70.72 ± 0.29; 70.36 ± 0.19) in respective species as shown in Table 4.

Discussion

Water quality parameters

Fish culture with combinations of cultured species, stocking densities and the quantity and quality of the nutrient inputs are highly influence the water quality parameters in the pond system [18]. In present experiment, low water temperature observed in substrate ponds, compared to control, this may be due to the shading effect by substrates. The transparency of water generally indicates the presence of natural food particles for fish and the productivity of the water body and the recommended

range is 15-40 cm [19]. In the present study, transparency was in the recommended range. The low transparency in substrate ponds may be due to the presence of substrate which leads to leaching of nutrients and production of planktons. Water pH in T_1 and T_2 than T_0 was mainly due to production of hydrogen ions because of high nitrification, due to nitrifying bacteria on substrate [20]. Banerjea stated that 5-7 ppm of dissolved oxygen of a water body is good for productivity and waters having dissolved oxygen below 5.0 ppm to be unproductive [21]. During the study period the DO concentration varied from 6.07–8.53 and initial DO depletion in all treatments may be due to the predominant heterotrophic food production which leads to the consumption of oxygen [22]. Also, the grazing activity induces water turbulence which helps to increase DO in water column [23]. The pH, dissolved oxygen observed during the study period was within suitable limit for tropical fish culture [24]. The high alkalinity value indicates higher nutrient turn over and productivity which were observed in ponds with substrates. It mainly caused by the effect of carbon dioxide released during decay of organic matter. The positive relationship was found between phytoplankton density and hardness, this might be the reason for high hardness in control group, as phytoplankton density was found more in control rather than substrate ponds [25].

Nitrifying bacteria are known to improve water quality by converting highly nitrogenous toxins such as ammonia and nitrite to nitrate [26]. The low concentration of ammonia-N and nitrite-N was observed in substrate-based treatments compared to the control may be due to the result of their nitrification by the microorganisms attached to the substrates, generating nitrate-N as the end product that helps in autotroph proliferation [27]. The preliminary increase of nitrite in all ponds attributes to oxidation of ammonia released from decomposition of manure added initially as reported [28]. The high concentration of nitrate in bagasse ponds was mainly due to leaching of nutrients from bagasse. Also, high densities of *Chlorophyceae* and *Euglenophyceae*, significantly increases nitrate level [29]. The phosphate phosphorus showed positive relationship ($r=0.826$) with alkalinity. Hoque revealed high phosphate-phosphorus content was mainly due to the periodical application of phosphate fertilizers [30]. However, increasing trend of PO_4 -P in substrate tanks was due to increase in the organic matter's mineralization rate due to the higher heterotrophic bacterial biomass [31].

Fish survival, growth and production

Significant higher growth was observed in T_2 for all three species. The highest final weight and survival of fish and prawn was due to positive effect of periphyton on water quality, as well as efficient nutrient cycling due to periodical fertilization. It was observed the high ($P<0.05$) growth parameters for *L. fimbriatus* in terms of final weight, weight gain (%) and biomass under Bagasse+Feed treatment [32]. The greater abundance of filamentous green algae and zooplankton might have caused higher growth of carnatic carp in substrate ponds. In addition to this, the presence of significantly high concentration of chlorophyll-a linked phytoplankton biomass in water and high load of nutrient rich organic debris in biofilm act as single cell protein source and also due to the low ammonia and nitrite nitrogenous waste [33, 34]. Periphyton algae on substrate help

to enhance nitrate level in water which is utilized by autotrophs and thus increases the primary productivity in the culture system [7]. The high fish production in T_2 was attributed to the microbial periphyton developed on substrate which acts as a food for zooplankton, fish and prawn. Likewise, periphyton substrates not only help to provide food for prawn but to minimize territoriality of freshwater prawn by providing extra shelter [35]. This might be the reason of significantly high survival and production of prawn in substrate ponds in current study. Similar results reported [36].

Carcass composition

At the end of experiment, fringed lipped carp, Carnatic carp and freshwater prawn from substrate ponds showed comparatively high protein, fat and ash percentage than that of initial values, whereas moisture found low. The findings were similar and decreased carcass moisture with increasing dietary protein was reported [37-39]. The higher protein and fat content in the carcass of the treated fish indicates that there was favorable effect of biofilm on the carcass composition of fish and prawn. The biodegradable substrates like bagasse having more fibre and rough surface area, which favored higher production of heterotrophic bacterial population along with high quantities of colonized phytoplankton and zooplankton which in turn helps for better growth and nutrition of fishes.

The results of the experiment showed that periphyton significantly improved water quality by lowering concentration of toxic ammonia and nitrite. Also, periphyton along with supplementary feed treatment helps to increase the protein percentage in the fish and prawn. Sugarcane bagasse can be used as substrate in the cultural systems for enhancement of health and growth of the fish with increasing the production which helps for sustainable, environment-friendly and cost effective practice for resource poor farmers.

Conclusion

The results of the experiment showed that periphyton significantly improved water quality by lowering concentration of toxic ammonia and nitrite. Also, periphyton along with supplementary feed treatment helps to increase the protein percentage in the fish and prawn. Sugarcane bagasse can be used as substrate in the cultural systems for enhancement of health and growth of the fish with increasing the production which helps for sustainable, environment-friendly and cost effective practice for resource poor farmers.

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