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EFFECTS OF A BIODEGRADABLE SUBSTRATE, SUGARCANE BAGASSE AND SUPPLEMENTAL FEED ON GROWTH AND PRODUCTION OF FRINGE-LIPPED PENINSULA CARP, *LABEO FIMBRIATUS* (BLOCH)

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Substrate-based farming practices are considered viable low-cost technologies as they help in sustainable aquaculture production. This investigation of 90-day duration was carried out in mud-bottomed cement tanks to study the effect of substrate and/or feed on growth and production of fringe-lipped peninsula carp, *Labeo fimbriatus*. The treatments consisted of sugarcane bagasse (T_1), sugarcane bagasse plus fish-meal based diet (T_2), and fish-meal based diet (T_3). The substrate addition affected water quality; it decreased total ammonia. Dissolved oxygen was low following manuring and introduction of the substrate, but improved subsequently. Total plate count of bacteria in water was higher in bagasse-based tanks; its value on bagasse was higher in T_2 treatment than in T_1. Fish growth and survival was the best in T_2, followed by T_3 and T_1 treatments. Carcass proximate composition improved in all the treatments on termination of the experiment. The results indicate that *L. fimbriatus* effectively utilizes biofilm grown on sugarcane bagasse and provision of substrate reduces the need for artificial feed.

Key words: substrate, biofilm, water quality, *Labeo fimbriatus*, growth

INTRODUCTION

Development of viable low-cost technologies and their application to current farming practices would help in enhancing aquaculture production. Substrate based aquaculture is one such technology that has generated a lot of interest in recent years (Wahab et al. 1999, Tidwell et al. 2000, Azim et al. 2001, Keshavanath et al. 2001). By providing organic matter and suitable substrates, heterotrophic food production can be increased several fold which in turn would support fish production. Substrates provide the site for epiphytic

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microbial production, consequently eaten by fish-food organisms and fish. Fish harvest microorganisms directly in significant quantities, either from microbial biofilm on detritus or from naturally occurring flocks in water column (Schroeder 1978). Provision of substrate would therefore, be useful for the growth of microbial biofilm. Apart from forming food for fish, biofilm improves water quality by lowering ammonia concentration (Langis et al. 1998, Ramesh et al. 1999). The present study was undertaken to assess the effect of sugarcane bagasse and an artificial diet—provided either alone or in combination—on the water quality and growth and production of the medium sized fringe-lipped peninsula carp, *Labeo fimbriatus* (Bloch, 1795), an indigenous fish, which is a good candidate species for farming due to its excellent meat quality and market price (Basavaraju et al. 1995).

MATERIAL AND METHODS

Experimental set up

The experiment was conducted over a period of 90 days in nine 25 m^2 (5 × 5 × 1 m) cement tanks with 15-cm soil base. All the tanks initially received 0.25 kg of quick lime and 2.5 kg of poultry manure. Water was filled to the tanks from a perennial well and a depth of 90 ±2 cm was maintained throughout the experimental period. Subsequently, poultry manure was applied at 0.3 kg per tank every 15 days. Sugarcane bagasse, procured locally, was sun dried and bundles were made using nylon rope; they were introduced into 6 of the 9 tanks randomly at the rate of 5 kg each, by suspending the bundles at regular distances from bamboo poles kept across the tanks. After 45 days, once again 1.25 kg of the substrate was supplemented to each of the designated tanks. Fingerlings of *Labeo fimbriatus* (av. wt. 2.5 g) were stocked at 40 per tank (16 000·ha^{-1}) two weeks after the addition of manure and substrate. No feed was provided to the fish in 3 of the substrate-added tanks (T_1), while a pelleted diet, formulated according to Varghese et al. (1976) (Table 1) was fed to the fish in the remaining 3 substrate-added tanks (T_2) and the other 3 tanks without substrate (T_3) at 5% body weight for the first 30 days and 2% thereafter, in two equal rations daily.

Table 1

Ingredient proportion and proximate composition of feed ($\bar{x}$ ±s.e.) (wet weight basis)*

Ingredient	%	Proximate composition	%
Fish meal	25	Moisture	7.29 ±0.13
Rice bran	40	Crude protein	28.17 ±0.66
Groundnut oil cake	25	Crude fat	3.15 ±0.08
Tapioca flour	10	Crude fibre	15.90 ±0.54
		Ash	13.80 ±0.62
		NFE	31.69
		Energy content [kJ·g^{-1}]	12.50

* Average of three values

Sampling

Sampling for water quality parameters, total plate count of bacteria (TPC) in water and substrate, and evaluation of the plankton and fish growth were carried out at 15-day intervals. Water temperature, dissolved oxygen, and pH were measured using a Horiba (Japan) water quality analyser (Model U 10). Free carbon dioxide and total ammonia were analysed following standard methods (Anonymous 1995). TPC in water was estimated on nutrient agar at room temperature by spread plate method. TPC on substrate was enumerated according to Anwar et al. (1992). A known quantity of substrate was collected and rinsed three times to remove loosely adherent cells. Then it was re-suspended in phosphate-buffered saline and vortexed for three minutes to dislodge the biofilm cells and TPC of the suspension estimated as no. per g of substrate. Plankton was collected from 40 l of water using a 60-μm net and numerical estimation done by the Direct Census Method using a Sedgewick Rafter Cell having 100 equal squares (Jhingran et al. 1969).

At least half the number of fish stocked were collected at each sampling and their individual total length and total weight taken. On termination of the experiment all the surviving fish were harvested, their length and weight noted. Specific growth rate was calculated using the formula:

$$\mathrm{SGR} = \frac{\ln \text{final weight} - \ln \text{initial weight}}{\text{experiment duration}} \times 100\ (\%\ \text{per day})$$

Biochemical analysis

Proximate composition of feed, initial and final carcass samples, and protein content of biofilm were analysed following AOAC (Anonymous 1975) procedures.

Statistical analysis

Comparison among different treatments was done by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test at $P < 0.05$ (Duncan 1955, Snedecor and Cochran 1968).

RESULTS

The overall average values of water temperature ranged from 26.99 (T_1) to 27.39°C (T_3), whereas pH varied from 6.10 to 8.0. Following the addition of manure, bagasse, and feed, there was a decrease in dissolved oxygen, but it improved subsequently; the highest average value of 5.46 ppm was recorded in T_2, followed by 5.30 ppm in T_1, and 5.07 ppm in T_3. Free carbon dioxide values showed an increasing trend over the experimental period in all the treatments. The average value of total ammonia was the lowest in T_1 (5.64 μg at. $N \cdot l^{-1}$) and the highest in T_3 (8.21 μg at. $N \cdot l^{-1}$) (Table 2).

Table 2

Average values of pH, dissolved oxygen, ammonia, and free carbon dioxide in different treatments (n = 3; $\bar{x}$ ±s.e.)

Parameter		Days 0	15	30	45	60	75	90	Overall average
pH	T_1	7.39	7.75	7.54	7.59	6.10	6.40	6.47	
	T_2	7.23	7.80	7.43	7.66	6.23	6.58	6.45	
	T_3	7.37	8.00	7.70	7.77	6.26	6.39	6.45	
O_2 (ppm)	T_1	3.52 ±0.62	5.98 ±1.07	4.93 ±1.79	4.62 ±0.99	5.32 ±0.82	5.81 ±0.25	6.95 ±0.12	5.30
	T_2	3.42 ±0.41	5.32 ±0.38	5.13 ±1.67	4.45 ±0.94	6.52 ±0.98	6.41 ±0.50	6.94 ±0.07	5.46
	T_3	3.49 ±0.40	5.30 ±0.50	4.69 ±1.95	4.89 ±2.03	5.32 ±0.68	5.54 ±0.33	6.23 ±0.22	5.07
NH_3-N ($\mu g \cdot l^{-1}$)	T_1	3.23 ±0.49	6.07 ±2.28	4.73 ±0.84	7.64 ±4.32	6.14 ±1.65	5.79 ±0.77	5.91 ±1.31	5.64
	T_2	4.03 ±0.92	7.20 ±1.97	6.72 ±2.17	7.95 ±2.68	6.92 ±1.84	6.34 ±0.35	5.72 ±0.39	6.41
	T_3	5.99 ±3.02	10.00 ±1.90	8.04 ±1.08	8.09 ±0.61	10.22 ±2.82	8.27 ±0.50	6.83 ±0.78	8.21
CO_2 (ppm)	T_1	1.33 ±1.22	1.87 ±1.67	1.07 ±0.46	3.73 ±0.46	4.53 ±0.46	4.80 ±0.80	4.27 ±0.46	3.09
	T_2	1.07 ±0.92	3.73 ±1.67	2.67 ±0.92	2.40 ±0.80	4.53 ±1.66	4.00 ±0.80	4.53 ±0.92	3.28
	T_3	1.06 ±0.92	2.67 ±1.85	0.80 ±1.39	2.67 ±0.46	4.80 ±0.80	4.80 ±0.80	4.80 ±0.80	3.09

Table 3

Total plate count of bacteria in water ($\times 10^4 \cdot ml^{-1}$) and on substrate ($\times 10^4 \cdot g^{-1}$) (n = 3; $\bar{x}$ ±s.e.)

		Days 0	15	30	45	60	75	90	Overall average
Water	T_1	0.20 ±0.03	0.85 ±0.68	1.41 ±0.76	1.21 ±0.29	1.10 ±0.22	1.09 ±0.29	0.91 ±0.40	0.97
	T_2	0.18 ±0.04	0.67 ±0.13	1.27 ±0.61	1.07 ±0.41	1.06 ±0.34	0.72 ±0.13	0.90 ±0.37	0.84
	T_3	0.19 ±0.04	0.72 ±0.19	1.21 ±0.69	0.89 ±0.19	0.85 ±0.16	0.83 ±0.15	0.79 ±0.15	0.78
Substrate	T_1	—	1.20 ±0.90	127.60 ±69.5	13.20 ±5.24	13.55 ±5.58	8.80 ±4.61	1.00 ±0.36	
	T_2	—	1.40 ±0.35	169.00 ±77.27	13.40 ±3.50	12.00 ±6.17	4.10 ±1.22	1.20 ±0.25	

The mean TPC in water was the highest in T_1 ($0.97 \cdot 10^4 \cdot ml^{-1}$) and lowest in T_3 ($0.78 \cdot 10^4 \cdot ml^{-1}$). The average TPC of bacteria on bagasse varied from 1.0 to $127.6 \cdot 10^4 \cdot g^{-1}$ in T_1 and 1.2 to $169.0 \cdot 10^4 \cdot g^{-1}$ in T_2 (Table 3). The average values of phyto- and zooplankton recorded in water were 30.2 and 23.57 (specimens per litre) in T_1, 27.14 and 13.43 in T_2, and 35.19 and 15.86 in T_3 treatments, respectively. The density of phyto- and zooplankton on the substrate was 14 and 28.66 in T_1 and 17.67 and 28.42 (specimens per cm^2) in T_2 treatments, respectively.

The growth of fish was the highest in T_2, followed by T_3 and T_1 treatments. SGR followed the growth trend, being the highest in T_2 treatment. Fish survival and production were the highest in T_2. Analysis of proximate composition of the fish carcass carried out initially showed 11.62% protein, and 0.96% fat. These values increased on completion of the experiment; feed treatment (T_3) had better impact on both the parameters (Table 4).

Table 4

Average weight (g), SGR (% per day), percentage survival, production ($g \cdot 25\ m^{-2} \cdot 90\ day^{-1}$), and proximate composition of *Labeo fimbriatus* in different treatments

	T_1	T_2	T_3
Initial weight	2.50	2.50	2.50
Final weight	13.53 ±0.79	18.35 ±0.34	15.86 ±0.43
SGR	1.88 ±0.06	2.21 ±0.02	2.04 ±0.02
Survival	83.33	93.33	82.50
Production	450.95	685.00	523.38
Proximate composition (wet weight basis)			
Moisture	75.94	77.75	75.31
Crude protein	12.96	12.65	13.15
Crude fat	3.69	2.14	4.27
Ash	4.10	4.13	4.09
NFE	3.31	3.33	3.18
Energy content $kJ \cdot g^{-1}$	4.65	4.01	4.89

DISCUSSION

Fertilization and artificial feeding are regularly practiced in semi-intensive fish culture, as they enhance growth and production. However, application of fertilizer and feed affects water quality, though not adversely. Decreased level of dissolved oxygen was recorded in the different treatments of this study following the addition of manure, substrate, and feed; this can be attributed to increased biological oxygen demand. Low dissolved oxygen is characteristic for water with predominantly heterotrophic food production which accounts for bulk of the oxygen consumption (Moriarty 1997). A similar observation has been reported by Ramesh et al. (1999) and Umesh et al. (1999) in substrate-installed tanks. The increase, noticed in carbon dioxide level with the progress of the experiment, could be related to the higher organic load. Total ammonia level in T_3 tanks was relatively higher than in substrate-based treatments. The bacterial biofilm developed on the substrate would have brought down ammonia concentration by nitrification (Langis et al. 1998, Ramesh et al. 1999, Umesh et al. 1999); this implies the usefulness of substrates in improving water quality in culture systems, by lowering ammonia concentration.

Nutrient enrichment of water is known to increase both the thickness and cellular density of bacterial biofilm. The higher bacterial density in water from bagasse-based tanks is due to the organic matter contributed by manure as well as bagasse. In addition, bacteria in the biofilm could have added free cells to the water. The comparatively low density of phyto- and zooplankton recorded in T_2 treatment may be due to better grazing as reflected by the higher growth of fish in this treatment.

Studies carried out in recent years (Ramesh et al. 1999, Umesh et al. 1999, Wahab et al. 1999, Thompson et al. 2000, Tidwell et al. 2000, Azim et al. 2001, Keshavanath et al. 2001) have shown that the various substrates used, contribute to growth and production of the different cultured species. In the present study, *L. fimbriatus* showed a 4-fold weight increase in substrate-based tanks over a period of 90 days, clearly indicating that it could effectively utilize biofilm grown on bagasse. The role of microbial biofilm directly as food to planktophagous fish has been emphasized by a number of authors (Schroeder 1987, Zhu et al. 1990). Bhatnagar and Karamachandani (1970) observed that fringe-lipped carp grazes on diatoms and algae that grow on submerged rocks and twigs. According to Talwar and Jhingran (1992), *L. fimbriatus* being a herbivore feeds on diatoms, green algae, blue-green algae, etc. The decrease recorded in TPC values after the 30^{th} day in the present experiment clearly points out to effective grazing by *L. fimbriatus*.

The growth performance of fish was the best in substrate + feed treatment, being significantly higher than in substrate-alone or feed-alone treatments; growth under T_2 treatment was higher by 35.62% and 15.86% over T_1 and T_3 treatments respectively. Fish growth in feed-alone treatment was 17.22 percentage points higher than in substrate-alone treatment. In feed-alone treatment (T_3) fish achieved a 5-fold weight increment over the experimental period. This superior performance as compared to T_1 treatment could be attributed to the nutritional quality of the diet employed; it had 28.17% protein as against 24.1% in biofilm. The significantly better growth of fish in the combination treatment indicates that the species can efficiently utilize natural (plankton + biofilm) as well as artificial diets when provided together.

The present results demonstrate that *L. fimbriatus* effectively utilizes biofilm grown on sugarcane bagasse and provision of a substrate reduces the need for artificial feed. Since biodegradable wastes have high C : N ratio and harbour higher periphytic biomass, they are better suited as substrates. Use of cheaper substrates like sugarcane bagasse can greatly improve economic viability of aquaculture.

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