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Research Note

Farming of *Peneaus vannamei* in Biofloc System: A Participatory Farmer Experiment

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Marketing is one of the major issues faced by finfish farmers, involved in farming in artificial tanks, whereas there is a well-established market for shrimps. In this context, an on-farm experiment of rearing high-value white-leg shrimp (*Peneaus vannamei*) was conducted. However, the study identified that *P. vannamei* farming using reconstituted saline water in biofloc systems faces many issues and high mortality rates. The production of shrimp was 5.1 kg/m^3 with sizes ranging from 10 gm to 24 gm. It was noticed that molting of the animals leaves behind moult shells that lead to the blockage of drains and thereby affect functioning of filters. Mass mortality observed in most demonstrations was mainly due to the accumulation of hydrogen sulfide, oxygen depletion, and incidence of black spot disease. The study concludes that rearing of *P. vannamei* in artificial tanks by biofloc system farming requires close monitoring, technical skill, adequate water availability, well-maintained drainage facilities, uninterrupted power supply and quality feed.

Small-scale fish farming in artificial tanks by various methods, *viz.,* re-circulatory aquaculture system (RAS), biofloc, aquaponics, etc., has become popular recently. The lack of suitable natural water resources paved the way for marginal farmers to venture into fish farming in artificial tanks. The *Tilapia nilotica* is the most widely farmed fish in such units due to its high growth rate and the possibility of harvesting in a short duration (Ponzoni et al., 2011). However, producing low-cost Tilapia from cost-intensive artificial units may not be economically viable. In this context, an on-farm experiment of rearing high-value white-leg shrimp *(P. vannamei)* as an alternative to low-cost Tilapia in artificial tanks was carried out. *P. vannamei* can fetch three times higher prices than Tilapia (Suresh, 2007). *P. vannamei* farming is not widespread in inland areas due to the need for saline water. Farming of *P. vannamei* in artificial saline water in tanks was attempted earlier by various researchers across the globe (Fleckenstein et al., 2022). *P. vannamei* culture will not grow well if the salinity is too low (< 4.0 ppt). At low saline conditions, the ionic composition of these waters is primarily deficient in several vital minerals, including potassium and magnesium (Roy et al., 2007; Zhu et al., 2004). Hence addition of minerals is critical. Water quality and ionic balance should be within limits to arrest blockages in physiological processes and productivity of *P. vannamei* (de Moura et al., 2021). The objective of the present study was to evaluate the practical and economic feasibility of *P. vannamei* farming in artificial tanks by biofloc method.

Four farmers (Table 1) from different parts of Ernakulam district, Kerala, India, who are sufficiently experienced in fish farming in artificial tanks, were selected for the on-farm experiment and denoted as F1, F2, F3 and F4.

The experiment was carried out in circular water tanks, four numbers, each 30,000 liter capacity measuring 6 meters in diameter and 4 feet in height, made of Polyvinyl chloride sheets spread over circular steel frames. Each tank was equipped with four air pumps capable of delivering air at a rate of 30,000 liters per minute for full-time aeration. An inverter system (1 KVA) was also set up to operate air pumps during power outages. The tanks were

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installed one foot above the ground level, and a drainage pipe was fixed at the center of tanks through which wastewater was collected and transferred to an HDPE settling tank of 200-liter capacity. Clear water from the settling tank was pumped back to the main tank using a submersible pump. Ultraviolet protection sheets with 80 percent light transmission were used over the tanks to reduce direct exposure to sunlight and rain, keeping the plankton growth and pH levels steady.

The water of 10 ppt salinity compatible for rearing of *P. vannamei* was prepared by adding salts and minerals to fresh water as per the ratio described in Table 1 (Roy et al., 2007). The mineral content of re-constituted culture water is presented in Table 2.

Table 1. Ratio of minerals added to prepare artificial saline water

Sl. No.	Mineral	Quantity, kg/1000 litre
1	Sodium chloride, $NaCl$	10.000
2	Magnesium chloride, $MgCl_2$	4.000
3	Calcium carbonate, $CaCO_3$	0.250
4	Sodium bi carbonate, $NaHCO_3$	0.065

Table 2. Mineral content of re-constituted culture water

Sl. No.	Mineral	mg per litre	
		Before re-constitution	After re-constitution
1	Calcium	120	143
2	Magnesium	422	436
3	Chloride	26	5196
4	Total iron	1.09	0.65
5	Phosphorous as P	1.09	10.5
6	Sodium	16	3072
7	Potassium	121	143

Biofloc technology utilizes the combined action of bacteria, algae, or protozoa under adequate aeration and the presence of probiotics to improve water quality by converting waste and dirt into flocs that can act as feed for shrimp. Adequate aeration was ensured at all times. *P. vannamei p*ost larvae (PL- 10) were stocked in tanks at 700 per 1000 liters.

Hatched Artemia nauplii were fed four times daily till the eighth day. Each feeding was followed by mixing the same amount of sugar in the tanks to ensure the carbon-nitrogen ratio of 15:1 to aid heterotrophic bacteria in the system. The sugar addition was reduced to 70 percent of the feed quantity from the second month onwards as the system commenced to take carbon from shrimp droppings. Effective microorganism (EM Solution Maple EM.1 Hariyali) reconstituted in freshwater at the ratio of 1:25 along with sugar (3 kg/25 liter water) was applied daily at the rate of 10 milliliters per 1000 liter.

Prawn feed supplement (Erawan G 500 CP India Pvt Ltd) at the rate of 2.5 gm per kilogram of feed and vitamin C at the rate of 2.5 gm per kilogram of feed were also provided weekly after 60 days of stocking. Feeding was followed as per the schedule detailed in Table 3.

Water ammonia, nitrate, and nitrite were measured using a water test kit (API® FRESHWATER MASTER TEST KIT). pH was tested using Universal indicator with a color card (Merck Universal Solution Indicator). Alkalinity was tested using a water test kit (AQUA SOL Alkalinity Test Kit). The settleable floc was quantified using Imhoff cones, in which samples from 1 L of water were allowed to deposit for 15 min, and then the settled volume was read (Avnimelech, 2007).

Water quality was monitored periodically and presented in Tables 4, 5, 6 and 7. Due to mass mortality in other locations, water quality till the end of the cropping period could be collected only from location F4. The pH did not significantly vary throughout the experimental period ($p > 0.05$) (Table 7). The pH ranged from 7.6 to 8.2. The total ammonia concentration was low at the start of the culture (0.25 mg L^{-1}), subsequently increased up to 1 mg L^{-1}, and further reduced to 0.25 mg L^{-1} in the later phase.

The safe limit of ammonia for *P. vannamei* juveniles in low saline water is 3.55–3.95 mg L^{-1} (Lin & Chen, 2001) which was maintained throughout the culture. Nitrite levels increased on the 40^{th} (160 mg L^{-1}) and 100^{th} (190 mg L^{-1}) days, which was managed by removing the slurry from the bottom and adding fresh water and probiotics. The initial biofloc density of 5 ml per liter increased to 10 ml per liter in 20 days and 15 ml per liter in the next 40 days and subsequently maintained throughout the culture. Water quality management is critical in biofloc aquaculture systems. A positive correlation (0.66)

Table 3. Feeding schedule

Sl. No.	Days	Feed	Protein (%)	Fat (%)	Feed quantity (percentage of body weight)
1	1-7	Artemia nauplii	60	8	1
		Slow sinking crumble	36	5	14
2	8-20	Sinking crumble	36	5	9
3	21-30	Sinking pellet, 1.0 mm	36	5	5
4	31-60	Sinking pellet, 1.2 mm	36	5	4
5	61-85	Sinking pellet, 1.6 mm	36	5	3
6	86-110	Sinking pellet, 1.8 mm	36	5	1.2

Sodium bicarbonate at the rate of 15 gm in 1000 liter was added periodically to retain Alkalinity above 180 ppm. One day starvation in a month method, as recommended by Yildirim et al. (2019), was adopted to improve the digestion process of shrimps.

Table 4. Water quality parameters in Location F1

Days	Ammonia (mgL^{-1})	PH	Nitrite (mgL^{-1})	Alkalinity (mgL^{-1})	Floc (mlL^{-1})
1	0.00±0.0	8.0±0.5	0	150±10	07±0.30
16	0.50±0.10	8.1±0.6	0	160±12	08±0.28

Table 5. Water quality parameters in Location F2

Days	Ammonia (mgL^{-1})	PH	Nitrite (mgL^{-1})	Alkalinity (mgL^{-1})	Floc (mlL^{-1})
1	0.25±0.2	8.0±0.5	0	140±12	05±0.2
10	0.25±0.1	8.1±0.6	0	150±14	08±0.8
20	0.30±0.3	8.2±0.5	0	160±20	11±1
30	0.50±0.2	8.1±0.6	0	160±15	12±2

was noted between the feed and sugar added (Table 8) as a carbon source to maintain the ammonia level under control. No significant difference in Alkalinity was noticed throughout the culture, and its values ranged from 160 mg L^{-1} to 190 mg-1. More moulted shells could be seen when alkalinity values were above 180 mg L^{-1}, a unique characteristic of crustaceans (Middlemiss, 2016). Very low alkalinity in intensive aquaculture systems may lead to slow postmoult calcification, increasing the risk of mortalities through cannibalism.

Mass mortality was observed on the 16th day in location F1. According to Li et al. (2017) one of the reasons for in-mass mortality is an accumulation of toxic substances like hydrogen sulfide in the culture ponds. Mass mortality occurred in location F2 on the 30^{th} day due to black spot disease. Disease incidence may reduce proper feed intake since the proper nutrient requirement at early stages is crucial for larvae survival (Vikas et al., 2012). Upon examination of samples, the presence of pathogenic bacteria *Photobacterium damselae* and *Vibrio tubiashii* were confirmed in the hepatopancreases of the infected animals. Similar mass mortality due to black spot disease (BSD) was earlier reported by Hu et al. (2019) in the case of cultured Pacific white shrimp (*P. vannamei*). In F3, mass mortality was also observed on the 97^{th} day due to oxygen depletion.

Table 6. Water quality parameters in Location F3

Days	Ammonia (mgL^{-1})	PH	Nitrite (mgL^{-1})	Alkalinity (mgL^{-1})	Floc (mlL^{-1})
1	0.20±0.1	8.0	0	140±10	05±1
10	0.30±00.2	8.2	0	150±10	08±1
20	0.20±0.1	8.1	0	170±10	12±2
30	0.25±0.2	8.1	0	160±20	11±1
40	0.25±0.3	7.9	80	170±10	11±1
50	0.50±0	7.8	0	160±15	11±2
60	0.20±0.3	8.1	0	160±12	15±2
70	0.25±0.2	7.9	0	170±20	14±2
80	0.25±0.3	7.9	0	180±10	14±1
97	0.40±0.4	7.0	0	180±10	15±1

Table 7. Water quality parameters in Location F4

Days	Ammonia (mgL^{-1})	PH	Nitrite (mgL^{-1})	Alkalinity (mgL^{-1})	Floc (mlL^{-1})
1	0.25±0.2	8.2±0.5	0	160±10	05±0.5
10	0.25±0.2	8.2±0.5	0	170±10	08±1
20	0.25±0.2	8.2±0.5	0	165±10	10±2
30	0.25±0.1	8.2±0.5	0	170±20	10±2
40	0.25±0.1	7.6±0.5	160±10	160±15	10±2
50	1.00±0.2	7.6±0.8	0	170±10	10±2
60	0.00±0.2	8.2±0.5	0	180±10	15±3
70	0.25±0.2	7.6±0.7	0	180±15	15±4
80	0.25±0.1	7.6±0.5	0	190±20	15±3
90	0.25±0.2	7.6±0.5	0	190±10	15±4
100	0.25±0.1	7.6±0.5	190±20	180±10	15±2
110	1.00±0.2	7.6±0.5	0	190±10	15±2

These locations were not included in recording production data.

Production of 153 kg (5.1 kg/m^3) was realized at F4 with an average of 101.5±59.5 kg. Size at harvest ranged from 10 gm to 24 gm with an average of 17.8±11.2 gm. The survival of shrimp ranged from 37 to 87 percent among the experimental tanks. Periodical harvesting of shooters reduces standing stock, facilitating rest growth faster. Hence partial harvesting was done in location F4 during 70th, 90th, 110th and 120th days. The average production cost was Rs.330/- per kg, and farmers could sell the produce at Rs.350/- per kg.

One of the issues that was faced, the moult shell left behind after moulting of the animals that lead to the blockage of drains and tamper functioning of filters. Physical removal of moult shells is impossible as it disturbs the animals affecting feed intake. Routine sludge removal is also very essential.

P. vannamei farming in artificial tanks requires close attention and technical skill. Critical factors are critical to adequate water availability, well-maintained drainage facilities, uninterrupted power supply and quality feed. Standardization of culture practices is required before recommending *P. vannamei* for farming in artificial tanks. Mass mortality was observed in experimental units due

Table 8. Quantity of feed and sugar administered

DOC	feed (gm)	sugar (gm)
1	170	180
10	440	360
20	1680	1250
30	1640	915
40	2380	1120
50	3280	1500
60	820	920
70	1150	1150
80	1350	1350
90	1650	1550
100	1000	980
110	540	1280

to the accumulation of hydrogen sulfide, oxygen depletion, and black spot disease incidence. Production of 5.1 kg/m^3 could be realized with sizes ranging from 10 gm to 24 gm with an average of 17.8±11.2 gm. The average production cost was Rs.330/- per kg. The moulting of the animals leaves behind moult shells that lead to the blockage of drains and tamper functioning of filters.

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