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Optimizing Rock Phosphate Application for Enhanced Productivity and Economic Returns in Indian Butter Catfish (*Ompok bimaculatus*) Nursery Rearing

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ABSTRACT

This study evaluated the effects of varying rock phosphate (RP) application rates (25, 50 and 75 kg/ha) compared to superphosphate (50 kg/ha) on the nursery rearing performance of Indian butter catfish (*Ompok bimaculatus*) in Tripura, Northeast India. The experiment was conducted in fifteen 0.02 ha ponds over a 30-day period. The 25 kg/ha RP treatment outperformed other treatments, maintaining optimal water quality (dissolved oxygen: 7.3 mg/L, pH: 8.4) and stimulating diverse plankton blooms (Shannon-Wiener index: Phytoplankton 2.75, zooplankton 2.16). This treatment enhanced microbial activity, with increased total heterotrophic bacteria (water: 7.6 × 10^5 CFU/mL, soil: 62.8 × 10^5 CFU/g) and phosphate solubilizing bacteria (29.6 × 10^4 CFU/g soil). Consequently, maximum fish growth (154.5 mg), survival (43.7%), fry production (74,000/0.02 ha), and net economic returns (Rs 327,725/ha) were achieved. These findings establish 25 kg/ha as the optimal RP application rate for sustainable nursery productivity of Indian butter catfish, offering practical implications for commercial farmers. The study demonstrates the potential of RP as an economically viable and environmentally sustainable alternative to conventional phosphatic fertilizers in aquaculture.

Keywords Rock phosphate, Pond fertilization, Catfish, Nursery rearing, Primary productivity, Water quality, Economic analysis.

INTRODUCTION

Aquaculture is vital for global food security, with catfish farming constituting a significant component in Eastern and Northeastern India (Debnath *et al.* 2020). Among catfish species, the Indian butter catfish (*Ompok bimaculatus*), commonly known as 'pabda,' has gained considerable attention due to its high market demand and favorable growth characteristics. Recent studies have underscored its potential for aquaculture and conservation (Biswas *et al.* 2023). Efforts to optimize farming practices for this species have included strategies such as maintaining optimum stocking densities (Debnath *et al.* 2015), refining species composition in carp-based composite culture systems (Debnath *et al.* 2020), and manipulating photoperiods to enhance growth performance and economic returns (Hoque *et al.* 2024), among

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other approaches.

In addition to their economic importance, bottom-dwelling fish like *O. bimaculatus* play a critical ecological role in aquaculture pond ecosystems, particularly in nutrient cycling. Through their bioturbation activities, these fish interact with pond sediments, mobilizing phosphorus bound in the soil and making it available to primary producers. This bottom-grazing behavior can mitigate nutrient loss, thereby improving pond productivity (Adámek and Marsálek 2013). Phosphorus, a key nutrient in freshwater ecosystems, often limits primary productivity in aquaculture systems. While natural pond productivity forms the foundation of fish nutrition in extensive and semi-intensive systems, phosphorus fixation in pond soils poses a challenge over time.

Traditionally, superphosphate fertilizers have been used to stimulate plankton growth in aquaculture ponds. However, this practice raises economic and environmental concerns, such as high costs and the risk of eutrophication (Adnan *et al.* 2020). As a sustainable alternative, rock phosphate—a naturally occurring mineral—has shown promise in addressing these issues (Khoshru *et al*. 2023). Recent research highlights the potential of rock phosphate solubilization by soil microorganisms, which can enhance nutrient cycling and bioavailability. Unlike the rapid nutrient release from superphosphate, rock phosphate's gradual release supports the establishment of beneficial microbial communities, creating a more balanced and sustainable nutrient profile in aquaculture systems (Bahman *et al*. 2023, Rafael *et al.* 2018).

This study aims to determine the optimal application rate of rock phosphate for catfish nursery rearing systems, evaluating its effects on water quality, soil fertility, plankton productivity, microbial dynamics, fish growth performance, and economic returns. By comprehensively assessing these parameters, the research seeks to provide insights into rock phosphate's potential as an economically viable and environmentally sustainable fertilization strategy for aquaculture.

MATERIALS AND METHODS

Experimental setup

The study was conducted in Tripura, India, utilizing 15 nursery ponds, each with an area of 0.02 ha and a depth of 1 meter. A Randomized Block Design was employed, comprising five treatments with three replications each. The treatments included a control group (C) with no phosphate fertilizer application, T1 with rock phosphate (RP) applied at 25 kg P/ha, T2 with RP at 50 kg P/ha, T3 with RP at 75 kg P/ha, and T4 with superphosphate (SSP) at 50 kg P/ha, which served as the standard recommendation.

Pond preparation and fertilization

All ponds were initially dewatered, dried, and refilled with freshwater. Lime was applied at 250 kg/ha in two doses: Before fish stocking and 7 days after. Cattle manure was added at 5 MT/ha in two doses: One week after lime application and 10 days after fish stocking. Nitrogen fertilizer (urea) was applied at 100 kg N/ha in two doses: One week after manure application and 2-3 days after the second manure dose.

Phosphate fertilizers (rock phosphate: 18.7% P_2O_5, superphosphate: 16% P_2O_5) were applied in two doses: The first dose 2-3 days after urea application and the second 1-2 days later. Both fertilizers were sourced from the agronomy section of the research center.

Fish stocking and feeding

After a week of fertilization, when the pond water turned greenish-brown with approximately 30 cm of plankton turbidity, each pond was stocked with Indian butter catfish spawn (locally sourced). A random sample of 100 spawn was measured to determine the average initial length (4.13 ± 0.12 mm) and weight (10.34 ± 0.41 mg). The stocking density was set at 1 million spawn/ha (20,000 spawn/0.02 ha pond).

To minimize cannibalism, 'hide outs' made of locally available materials were provided in the ponds. Fish were fed a supplementary diet consisting of rice polish and mustard oil cake (1:1 ratio). The feeding rate was set at 6 kg per million spawns per day for the first 5 days, and then increased to 12 kg per million spawns per day thereafter. The daily ration was divided into two equal portions and provided at

09:00 and 16:00 hrs (Ayyappan *et al.* 2019).

Water quality analysis

Water samples were collected weekly between 09:00 and 10:00 hrs for the analysis of various physico-chemical parameters. Temperature was measured using a mercury thermometer, and transparency was assessed with a Secchi disc. Dissolved oxygen levels were determined using Winkler's method, while free CO_2 and total alkalinity were analyzed through titrimetric methods. The pH was measured using a digital pH meter, nitrate-N was estimated spectrophotometrically, and phosphate-P was analyzed using the stannous chloride method. All analyses were carried out following the standard protocols outlined in APHA (2017).

Plankton analysis

Plankton samples were collected weekly by filtering 50 liters of water through a plankton net (60 μm mesh size) from each pond. The concentrated samples were preserved in 4% formalin. Plankton were identified to genus level using standard keys and quantified using a Sedgewick-Rafter counting cell. Plankton diversity was assessed using the Shannon-Wiener index ($H' = -\Sigma Pi(lnPi)$), where Pi is the proportion of each species in the sample.

Primary productivity and chlorophyll-a

Primary productivity was estimated weekly using the light and dark bottle method. Two light bottles and one dark bottle were suspended at mid-depth in each pond for 4 hrs (10:00-14:00). Dissolved oxygen was measured in initial and final samples, and gross primary productivity (GPP) and net primary productivity (NPP) were calculated.

Chlorophyll-a content was analyzed weekly by filtering 500 ml of water through Whatman GF/C filter paper, extracting pigments with 90% acetone, and measuring absorbance using a spectrophotometer at 630, 645 and 665 nm wavelengths.

Soil analysis

Soil samples were collected weekly from five sites in each pond (four corners and center) to a depth of 10 cm. Composite samples were air-dried, ground, and sieved through a 2 mm mesh. Available nitrogen was determined by the alkaline permanganate method, available phosphorus by Olsen's method, and organic carbon by Walkley and Black's rapid titration method (Saha 2010).

Microbial analysis

Total heterotrophic bacteria (THB) counts in water and soil were determined weekly using the spread plate method on nutrient agar. Water samples were serially diluted, and 0.1 ml of appropriate dilutions was spread on agar plates. For soil, 10 g of soil was homogenized in 90 ml of sterile saline, serially diluted, and plated. Plates were incubated at 30°C for 48 hrs before counting.

Phosphate solubilizing bacteria (PSB) were enumerated using Pikovskaya's agar. Soil dilutions were spread on plates and incubated at 30°C for 7 days. Colonies showing clear zones were counted as PSB.

Soil phosphatase activity was determined using p-nitrophenyl phosphate as a substrate. The release of p-nitrophenol was measured under acidic (pH 6.5) and alkaline (pH 11) conditions. The enzyme activity was expressed as μg p-nitrophenol released per gram of soil per hour.

Fish growth and survival

After 30 days of rearing (nursery phase), all ponds were completely harvested by repeated netting followed by draining. Total number and weight of surviving fry were recorded. A random sample of 100 fry from each pond was measured for individual length and weight. The following parameters were calculated:

Weight gain (mg) = Final weight - Initial weight
Survival rate (%) = Number of harvested fry / Number of stocked spawn × 100
Fry production = Total number of fry harvested per pond.

Economic analysis

An economic analysis was conducted using prevailing market prices for all inputs (feed, fertilizers, labor) and fish fry. Gross revenue, net revenue, and benefit-cost ratio (BCR) were calculated for each treatment.

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test to determine significant differences among treatments. Statistical significance was set at $p<0.05$. All statistical analyses were performed using SPSS software version 21.0.

RESULTS AND DISCUSSION

The study revealed significant variations in water quality, soil fertility, plankton dynamics, microbial activity, and fish growth across different rock phosphate (RP) application treatments (Tables 1–8).

The 25 kg/ha RP treatment (T1) exhibited superior water quality parameters, with significantly higher dissolved oxygen (7.3 ± 0.5 mg/L) and pH (8.4 ± 0.3) compared to other treatments (Table 1). These values align with optimal ranges for fish culture, creating conducive conditions for plankton growth (Hoque *et al.* 2024). Phosphate-P levels in T1 were comparable to superphosphate treatment, indicating that lower RP application rates can maintain optimal phosphorus levels.

Soil analysis showed that T1 significantly increased available soil phosphorus (29.7 ± 3.2 kg/ha) compared to the control, without substantially altering nitrogen or organic carbon content (Table 2). This gradual phosphorus release characteristic suggests potential long-term benefits for soil fertility management in aquaculture ponds (Bahman *et al.* 2023).

The 25 kg/ha RP treatment supported significantly greater plankton abundance (27,600 ± 3,200 cells/L) with maximum phytoplankton (H' = 2.75 ± 0.31) and zooplankton (H' = 2.16 ± 0.27) diversity indices (Table 3). This diverse plankton community provides a nutritionally balanced natural food source, potentially reducing reliance on supplementary feeds (Biswas *et al.* 2023).

Primary productivity parameters were significantly enhanced in T1, with higher chlorophyll-a concentrations (62.4±7.5 µg/L), gross primary productivity (6.31 ± 0.68 mgC/L/hr), and net primary productivity (4.29 ± 0.55 mgC/L/hr) compared to

Table 1. Water quality parameters (mean ± SD) during nursery rearing. Means with different superscript letters in the same row are significantly different ($p<0.05$).

Parameter	Control	25 kg/ha RP	50 kg/ha RP	75 kg/ha RP	50 kg/ha SP
Temperature (°C)	28.4±1.8^{a}	27.8±1.5^{a}	28.6±1.3^{a}	28.2±1.6^{a}	28.1±1.7^{a}
Transparency (cm)	32.8±5.2^{a}	28.5±4.1^{b}	30.7±4.8^{ab}	29.1±4.5^{b}	31.4±4.9^{ab}
DO (mg/L)	5.9±0.6^{c}	7.3±0.5^{a}	6.5±0.7^{b}	6.9±0.6^{ab}	7.1±0.5^{ab}
Free CO_2 (mg/L)	7.1±1.4^{a}	6.2±1.1^{a}	6.8±1.5^{a}	6.4±1.3^{a}	6.6±1.2^{a}
pH	7.5±0.4^{c}	8.4±0.3^{a}	8.1±0.4^{ab}	8.5±0.5^{a}	8.3±0.4^{a}
Total alkalinity (mg/L)	124±15^{a}	131±18^{a}	128±13^{a}	125±16^{a}	127±14^{a}
NO_3-N (mg/L)	0.25±0.07^{a}	0.21±0.05^{a}	0.27±0.08^{a}	0.23±0.06^{a}	0.24±0.07^{a}
PO_4-P (mg/L)	0.21±0.05^{d}	0.74±0.12^{b}	0.59±0.09^{c}	0.91±0.16^{a}	0.81±0.14^{ab}

Table 2. Soil fertility parameters (mean ± SD) at end of nursery rearing of fish (one month). Means with different superscript letters in the same column are significantly different ($p<0.05$).

Parameter	Control	25 kg/ha RP	50 kg/ha RP	75 kg/ha RP	50 kg/ha SP
Avail N (kg/ha)	31.5±3.4^{a}	32.8±4.1^{a}	30.6±2.9^{a}	29.8±3.7^{a}	31.1±3.2^{a}
Avail P (kg/ha)	17.4±2.1^{c}	29.7±3.2^{a}	25.3±2.6^{b}	22.1±2.4^{bc}	31.4±2.8^{a}
Org C (%)	0.79±0.13^{a}	0.74±0.09^{a}	0.81±0.15^{a}	0.76±0.11^{a}	0.72±0.08^{a}

Table 3. Plankton diversity (mean ± SD). Means with different superscript letters in the same column are significantly different (p<0.05).

Treatment	Phytoplankton H'	Zooplankton H'
Control	1.82±0.24^{c}	1.27±0.19^{b}
25 kg/ha RP	2.75±0.31^{a}	2.16±0.27^{a}
50 kg/ha RP	2.41±0.28^{ab}	1.84±0.23^{ab}
75 kg/ha RP	2.59±0.33^{ab}	2.05±0.30^{a}
50 kg/ha SP	2.28±0.26^{b}	1.72±0.19^{ab}

Table 4. Primary productivity parameters (mean ± SD). Means with different superscript letters in the same column are significantly different (p<0.05).

Treatment	Chl-a (µg/L)	Gross PP (mgC/L/hr)	Net PP (mgC/L/hr)
Control	28.6±4.8^{c}	2.82±0.41^{c}	1.57±0.32^{c}
25 kg/ha RP	62.4±7.5^{a}	6.31±0.68^{a}	4.29±0.55^{a}
50 kg/ha RP	48.9±6.2^{b}	5.17±0.74^{b}	3.11±0.49^{b}
75 kg/ha RP	54.3±6.9^{ab}	5.64±0.81^{ab}	3.48±0.61^{b}
50 kg/ha SP	46.5±5.7^{b}	4.95±0.65^{b}	2.97±0.43^{b}

Table 5. Total heterotrophic bacteria counts (mean ± SD). Means with different superscript letters in the same column are significantly different (p<0.05).

Treatment	Water (×10^{5} CFU/mL)	Soil (×10^{5} CFU/g)
Control	2.8±0.5^{c}	28.4±4.2^{c}
25 kg/ha RP	7.6±1.1^{a}	62.8±7.5^{a}
50 kg/ha RP	5.9±0.9^{b}	48.6±6.3^{b}
75 kg/ha RP	6.5±1.2^{ab}	54.7±6.9^{ab}
50 kg/ha SP	5.2±0.7^{b}	42.1±5.8^{b}

other treatments (Table 4). This suggests that the gradual phosphorus release from rock phosphate creates more favorable conditions for sustained phytoplankton growth (Adnan *et al.* 2020).

Microbial populations showed significant variations across treatments. T1 exhibited the highest total heterotrophic bacterial counts in water (7.6 ± 1.1 ×10^{5} CFU/mL) and soil (62.8 ± 7.5 × 10^{5} CFU/g), with peak phosphate-solubilizing bacteria (29.6 ± 4.2 × 10^{4} CFU/g soil) (Tables 5–6). Enhanced microbial activity can improve nutrient cycling, potentially reducing fertilizer application frequency (Sahu and Jana 2000).

Table 6. Phosphate solubilizing bacteria counts (mean ± SD). Means with different superscript letters in the same column are significantly different (p<0.05).

Treatment	PSB counts (×10^{4} CFU/ g soil)	Acid phosphatase (µg p-nitrophenol/ g/hr)	Alkaline phosphatase (µg p-nitrophenol/g/hr)
Control	8.3±1.6^{c}	184±28^{c}	122±18^{b}
25 kg/ha RP	29.6±4.2^{a}	392±46^{a}	295±32^{a}
50 kg/ha RP	21.5±3.8^{b}	306±39^{b}	226±25^{a}
75 kg/ha RP	25.7±3.9^{ab}	342±42^{ab}	251±29^{a}
50 kg/ha SP	17.4±2.5^{b}	279±35^{b}	197±21^{a}

The 25 kg/ha RP treatment demonstrated superior fish growth performance, with maximum weight gain (154.5 ± 18.4 mg), survival rate (43.7 ± 6.2%), and fry production (74,000 ± 7,400 fry/0.02 ha pond) (Table 7). This performance surpassed the superphosphate treatment, highlighting rock phosphate's potential in catfish nursery rearing (Debnath *et al.* 2022).

Economic analysis revealed that T1 yielded the highest net returns of Rs 327,725 per hectare, with the highest benefit-cost ratio (2.00) (Table 8). This economic advantage stems from increased fry production, lower fertilizer costs, and improved feed efficiency (Jena *et al.* 2020).

The findings demonstrate rock phosphate's potential as an economically viable and environmentally sustainable alternative to conventional phosphatic fertilizers in catfish nursery ponds. By optimizing water quality, enhancing plankton productivity, stimulating beneficial microbial communities, and improving fish growth and survival, rock phosphate application at 25 kg/ha offers a promising strategy for sustainable aquaculture intensification.

This study highlights the potential of rock phosphate as a cost-effective and sustainable alternative to conventional fertilizers in catfish nursery ponds. Applying rock phosphate at 25 kg/ha in two split doses during pond preparation for *Ompok bimaculatus* nurseries can reduce fertilizer costs while enhancing productivity. The application improves water quality, boosts plankton productivity, supports beneficial microbial communities, and enhances fish

Table 7. Growth performance and survival (mean ± SD). Means with different superscript letters in the same row are significantly different ($p<0.05$).

Parameter	Control	25 kg/ha RP	50 kg/ha RP	75 kg/ha RP	50 kg/ha SP
Initial wt (mg)	10.2±1.6[a]	10.5±1.8[a]	10.1±1.5[a]	10.6±1.7[a]	10.3±1.4[a]
Final wt (mg)	72±8[c]	165±19[a]	122±15[b]	132±17[b]	115±13[b]
Wt gain (mg)	61.8±7.2[c]	154.5±18.4[a]	111.9±13.8[b]	121.4±15.6[b]	104.7±12.1[b]
Survival (%)	30.2±5.4[c]	43.7±6.2[a]	37.6±5.9b[c]	39.8±5.3[ab]	35.5±6.4[c]
Fry produced	30,200±5100[c]	74,000±7400[a]	37,600±7100[bc]	39,800±?	35,500±6400[ab]

Table 8. Estimated cost and benefit (Rs) from different treatments over a one-month period in a one-hectare pond. Note: SSP: Rs 15/kg, rock phosphate: Rs 10/kg, Urea: Rs 5/kg, Fish fry: Rs 1/seed (<100 mg) and Rs 1.5/seed (>100 mg) as fixed by the institute, 100 cc of fish spawn: Rs 500 (100 cc contains approximately 25,000 spawns), Feed: Rs 30/kg.

Items	Control	25 kg/ha RP	50 kg/ha RP	75 kg/ha RP	50 kg/ha SP
A. Costs					
Pond lease (ha/year)	10,000	10,000	10,000	10,000	10,000
Pond clearance	5,000	5,000	5,000	5,000	5,000
Lime	5,000	5,000	5,000	5,000	5,000
Manure	10,000	10,000	10,000	10,000	10,000
Fertilizers	1,100	2,440	3,780	5,110	6,100
Fish seed	20,000	20,000	20,000	20,000	20,000
Feed	139,739	271,335	208,815	188,436	151,690
Labor	3,000	3,000	3,000	3,000	3,000
Miscellaneous	1,000	1,000	1,000	1,000	1,000
Total	194,839	327,775	266,595	247,546	211,790
B. Gross benefits	302,000	655,500	474,700	425,800	351,500
C. Net benefits (B-A)	107,161	327,725	208,105	178,254	139,710
D. BCR	1.55	2.00	1.78	1.72	1.66

growth and survival. Although limited to a 30-day nursery phase, the findings underscore its promise for sustainable aquaculture intensification. Long-term studies encompassing rearing and grow-out phases are recommended to fully understand its cumulative benefits on soil fertility and pond productivity, offering valuable insights for commercial catfish farmers.

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