**Assessment of Carbon budget under semi-intensive rearing of Reba, *Cirrhinus reba* (Hamilton, 1822) with varying stocking density**

**Abstract**

A carbon budget was assessed for the rearing of *Cirrhinus reba* in 9 tanks for 45 days. Each tank was with an equal amount of 20 m2 (5m ×4m ×1m) water area. Three various Stocking densities are designed and named as *@*RS1: 1.0, RS2: 2.0 and RS3: 3.0 lakh fry per hectarewhen RS is Reba stocking. Fish fry was fed with a 30% protein diet, and it was formulated by using various ingredients, viz. soybean meal, mustard oil cake, fish meal, wheat bran, broken wheat, corn flour and vitamin-mineral mixture in appropriate proportion. The tanks were fertilized uniformly by applying lime at 300 kg ha-1 and mustard oil cake at 750 kg ha-1. The recovery of carbon in fish was highest at 27.84% in RS2, followed by RS1 (25.07%) and RS3 (22.99%). At the end of the experiment, discharged water accounted for 43.03- 52.48% OC (organic carbon) of the total inputs. The varying stocking density affected the accumulated organic carbon in the discharged water (P<0.05). The experiment suggests that carbon utilization will be better at the desirable stocking density, @ 2.0 lakh per hectare, for a semi-intensive culture system of reba carp.

**Keywords:** Reba,Organic Carbon, Growth, Stocking Density.

**Introduction**

Aquaculture is defined as the "planned and purposeful production of aquatic animals and plants, or both together." In recent years, there has been an increased focus on diversifying culture systems, leading to the incorporation of various potential species, particularly minor carps, into traditional carp culture systems. Examples include species like *Labeo calabasu*, *L. bata*, and *L. gonius*. Similarly, the Reba carp (*Cirrhinus reba*) has been identified as a promising candidate species for culture, particularly in semi-intensive aquaculture systems in many Southeast Asian countries. Recently, *C. reba* has gained attention as a potential new species for aquaculture. *Cirrhinus reba* contributes significantly to capture fisheries in the River Ganga and, historically, also in the River Narmada (Sarkar et al., 2004). Recognized as an important food fish (Jhingran, 1991; Rahman, 2005), *C. reba* is a valuable source of protein, calcium, and low-fat content, making it an ideal dietetic food for human consumption (Afroz and Begum, 2014). The culture of aquatic animals involves a variety of inputs, including manures, fertilizers, feed, and combinations thereof. In a pond ecosystem, nutrients are primarily distributed across the water, fish biomass, and pond sediment.

The sediment in pond ecosystems plays a crucial role in maintaining the balance of the culture system, acting as a buffer for nutrient concentrations present in the water (Chien and Lai, 1988). According to Avnimelech et al. (1984), even a thin sediment layer just a few centimeters deep can hold more nutrients than the water column itself. It is commonly believed by fish farmers that a significant portion of the nutrients introduced into ponds ends up in the pond's sediment and is later discharged as effluent. Estimating the nutrient budget is essential to understanding how added nutrients are utilized within the system and to minimize nutrient losses to the surrounding water. A carbon budget can help assess the potential pollution impact of specific pond management strategies.

Nutrient budgets have been studied in various aquaculture systems, including those for channel catfish (Boyd, 1985), striped bass (Daniels and Boyd, 1989), tilapia (Green and Boyd, 1995), high-density shrimp farming (Hopkins et al., 1993; Martin et al., 1998), Indian major carps (Adhikari et al., 2014), reba carp (Keer et al., 2018b), and shrimp (Sahu et al., 2013). However, nutrient budget data for Indian major carps and minor carp culture remain limited. Thus, the current study aims to assess the organic carbon (OC) uptake by Reba fry at various stocking densities.

**Materials and methods**

**Experimental site and design**

The study was conducted in cement cistern tanks (Image 1.), each with a volume of 20 m³ (5m x 4m x 1m), located at the Department of Aquaculture, College of Fisheries, Lembucherra, Tripura, India. The experiment was carried out during the monsoon season from 15 July to 30 August 2016. A completely randomized design (CRD) was used, and the tanks were divided into three treatment groups: RS1, RS2, and RS3, each with three replications. The stocking densities for Reba carp were set at 1 million (RS1), 2 million (RS2), and 3 million (RS3) per hectare. The duration of the experiment was 45 days.

**Image 1. Experimental site (Left) and A harvested haul of Reba (Right)**

**Tank preparation, fertilisation and stocking**

The tanks were provided with an 8 cm thick layer of red soil. Prior to the experiment, the tanks were exposed to sunlight. Standard management practices for carp rearing were followed in preparing the tanks (Jhingran, 1991; Mohanty et al., 1993). The tanks were first cleaned, dried, and treated with lime (100 g Ca(OH)₂ per tank) at a rate of 300 kg per hectare, then filled with groundwater. One week after liming, the tanks were fertilized with mustard oil cake at 750 g per tank, applied in two split doses (750 kg per hectare), to promote plankton production. The oil cake was soaked in water for 24 hours before application. Manuring and fertilization were carried out at regular intervals, both before and after stocking the fry, in line with the carp culture management practices to enhance production (Anon, 1985). The *C. reba* seeds were sourced from the Hatchery situated at the Department of Aquaculture, College of Fisheries, Lembucherra, Tripura, India, and were acclimatized before stocking, which occurred in the morning. The experimental tanks were stocked with fry having an initial length of 3.81 cm and weight of 0.59 g

**Diet preparation and feeding**

The diet for the experimental fry was formulated to contain 30% protein, using a mix of soybean meal, mustard oil cake, fish meal, wheat bran, broken wheat, corn flour, and a vitamin-mineral mixture in the appropriate proportions (Table 1) (Keer et al., 2018a). The fry were initially fed twice daily at a rate of 6-8% of their body weight. The amount of feed was adjusted every 15 days based on the increase in the average body weight. The feed was provided in pellet form.

**Table 1. Inclusion of various ingredients**

|  |  |
| --- | --- |
| Ingredients |  Inclusion (g/kg) |
| Soybean meal | 150 |
| Mustard oil cake | 300 |
| Fish meal | 200 |
| Wheat bran | 150 |
| Broken wheat | 100 |
| Corn flour | 98.50 |
| Vitamin-mineral mixture | 1.50 |

**Growth performance**

At the end of the experiment, growth in terms of weight was recorded, and specific growth rate (SGR) and survival (%) were calculated with the following formulae-

SGR (%) = $\frac{In (Final wet body weight) – In (Initial wet body weight) }{Time duration (days)}×100$

Survival (%) = $\frac{Number of final harvest }{Total number stocked}×100$

**Carbon budget**

 Total organic carbon (OC) was calculated based on the input, output, uptake, and accumulation in the culture system during the experimental period using the following method.

**Organic carbon (OC) in soil sediment**

 Total OC of the soil and in the fish was determined using rapid titration method (Walkley and Black, 1934).

**Organic carbon (OC) in Water**

 The total organic carbon in tank water was determined by using chromic acid rapid titration method (APHA, 2005).

**Organic carbon input in the form of feed and fish was calculated as follows (**Mohanty et al., 2009**):**

Organic carbon (OC) in feed= OC concentration in feed × total amount of feed supplied.

Organic carbon (OC) in fish =OC concentration in fish carcasses × total fish biomass

**Physicochemical parameters of water**

Different water quality parameters viz. Water temperature (0C), pH, DO (mg l-l), NO3-N (mg l-l) and PO4-P (mg l-l) were determined during the experimental period using standard APHA methods (2005).

**Analytical method and statistical analysis**

The data obtained were statistically analysed using one-way analysis of variance (ANOVA) and interpreted through appropriate statistical methods with the Statistical Package for Social Sciences (SPSS, version 16.0 for Windows). Duncan's New Multiple Range Test was applied to determine if significant differences existed among treatment means (Duncan, 1955; Zar, 1999). A P-value of < 0.05 was considered statistically significant in all cases.

**Results and Discussion**

The final mean weight of the Reba was significantly (*p*<0.05) affected by varying stocking density, as shown in Table 2. Similarly, SGR and survival (%) were also found in the same trend. The higher weight gain was recorded in RS1 (6.62±0.04) and followed by RS2 (5.00±0.18) and RS3 (2.70±0.09). The higher SGR was also recorded in RS1 (5.38±0.01) and followed by RS2 (4.75±0.08) and RS3 (3.38±0.07). Similarly, higher survival was recorded in RS1 (88.33±1.45), followed by RS2 (79.83±2.01) and RS3 (69.56±1.56).

**Table 2. Growth parameter during the rearing of Reba for carbon assessment in the semi-intensive system**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Experimental Groups** | **Initial weight (Mean)** | **Final Weight (Mean)** | **SGR (%)** | **Survival (%)** |
| **RS1** | 0.59 | 6.62±0.04c | 5.38±0.01c | 88.33±1.45c |
| **RS2** | 0.59 | 5.00±0.18b | 4.75±0.08b | 79.83±2.01b |
| **RS2** | 0.59 | 2.70±0.09a | 3.38±0.07a | 69.56±1.56a |

\*Data expressed as mean ± SE, n=3

\*Data in same column with different superscripts are significantly (*p*<0.05) differ.

Organic carbon input, output and accumulation of the carbon in the experimental tank are depicted in Table 3. The total input of OC (g) was calculated (*p*<0.05) and found lower in RS1 (411.50±5.46) and Followed RS3 (548.42±2.04) and RS2 (559.41±6.39).The total OC (g) output through fish was found significantly (*p*<0.05) lower in RS1 (103.13±.68), RS3 (126.04±3.98) and RS2 (155.80±5.78). The OC (g) output in the form of discharged water from the tanks of each group did not differ significantly(*p*<0.05), the value was lower in RS1 (114.66±8.97) followed by RS2 (117.87±7.74) and RS3 (123.20±1.24).The total OC (g) output was found significantly (*p*<0.05) lower in RS1 (217.80±9.49), RS3 (249.24±1.28) and RS2 (273.67±2.69), RS1 and RS3 did not differ (*p*<0.05) significantly with RS2.

**Table 3: Organic carbon (OC) assessment of rearing of reba fry**

|  |  |  |
| --- | --- | --- |
| **Parameters** | **Organic carbon (g)** | **Organic carbon (%)** |
| **T1** | **T2** | **T3** | **T1** | **T2** | **T3** |
| **Input** | **Feed** | 208.94±3.22a | 349.38±7.38b | 339.99±1.99b | 50.77±0.20a | 62.44±0.61b | 61.89±1.39b |
| **Water** | 70.40±2.44a | 77.86±1.41ab | 76.26±1.06b | 17.098650.39b | 13.93±0.37a | 13.94±0.47a |
| **Fertilizer (MOC)** | 132.16±0.0 | 132.16±0.0 | 132.16±0.0 | 32.13±0.43b | 23.63±0.27a | 24.17±0.93a |
| **Total input** | 411.50±5.46a | 559.41±6.39b | 548.42±2.04b | 100.00 | 100.00 | 100.00 |
| **Output** | **Fish** | 103.13±.68a | 155.80±5.78b | 126.04±3.98c | 47.52±1.88a | 56.97±2.52b | 50.80±2.82ab |
| **Water** | 114.66±8.97a | 117.87±7.74a | 123.20±1.24a | 52.48±1.88b | 43.03±2.52a | 49.20±2.82ab |
| **Total output** | 217.80±9.49a | 273.67±2.69ab | 249.24±1.28b | 100.00 | 100.00 | 100.00 |
| **Nutrient retention** | 193.70±4.15a | 285.74±7.98b | 299.18±2.41b | 47.11±1.63a | 51.06±0.88ab | 54.42±2.84b |
| **Sediment accumulation** | 122.93±1.7a | 133.57±6.30a | 145.99±1.88a | 29.99±4.61a | 23.88±1.15a | 26.79±3.82a |
| **Others** | 70.78±1.32a | 152.17±8.24a | 153.19±4.07a | 17.12±3.04a | 27.18±1.22a | 27.64±6.67a |
| **Nutrient recovered by fish** | 103.14±0.68a | 155.8±5.78b | 126.04±7.88c | 25.07±0.20b | 27.84±0.72c | 22.99±0.24a |

**\*Data expressed as mean ± SE, n=3**

**\*Mean value in the same row with different superscripts vary significantly (*p*<0.05)**

Significantly, higher OC (%) recovery was seen in the RS2 (56.97±2.52) as compared to other groups (RS1;50.80±2.82 and RS3;47.52±1.88).Similarly, the recovery of OC (%) was higher in RS2 (43.03±2.52), followed by RS1 (52.48±1.88)and RS3 (49.20±2.82). Different water quality parameters did not differ significantly (*p*<0.05) among the treatments. The values of all the water quality parameters are presented in Table 4.

**Table 4: Ranges of different physicochemical parameters of water**

|  |  |
| --- | --- |
| **Parameters (units)** | **Ranges** |
| Water temperature (0C) | 28.43-33.10 |
| pH | 7.89- 8.17 |
| DO (mg l-l) | 7.20 to 10.0 |
| NO3-N (mg l-l) | 0.18-0.18 |
| PO4-P (mg l-l) | 0.05-0.62 |

Aquaculture is well established, and the stoking density is considered a critical factor in a novel aquaculture system. The stocking density affects the whole ecosystem as well as available nutrients and their utilisation in the system, and it is directly related to the competition for food, space and nutrient utilisation. Therefore, the present study aimed to assess the carbon utilisation in a semi-intensive culture system. The growth and survival of fish in the current study are affected (*p*<0.05)by the different stocking densities (Table 1).

Sediment plays a key role in regulating the nutrient dynamics of an aquaculture system by buffering the concentration of nutrients in the water (Chien and Lai, 1988). Avnimelech et al. (1984) noted that a small sediment layer of only a few centimetres could store more nutrients than the water column above it. Therefore, it is necessary to access the nutrient budget in the culture system. The recovery in organic carbon (OC) was affected (*p*<0.05) with varying stocking densities in the current study. The higher growth (RS1; 1lakh Reba fry per ha.) might be due to less competition at lower crowding in the system. Higher growth of fish at lower stocking densities was recorded by numerous scientists, who stated that the high densities create stressful situations in the rearing system with the presence of abundant food interaction (Houde, 1975; Haque et al., 1994; Rahman and Rahman, 2003; Onxayvieng et al., 2021; Li et al., 2024). The higher recovery of OC (%) in fish (RS2; 2 lakh per hectare) might be due to the better utilisation of organic carbon as well as balanced nutrients in the ecosystem. The present study corroborates the earlier reported study (Adhikari et al., 2014; Keer et al., 2018b). The physicochemical parameters of water were not affected by the various stocking densities. And found to be congenial to fish growth. Similar values were also found in various fish rearing and culture systems (Rahman and Rahman, 2003; Rahman et al., 2005), where it was stated that these ranges are desirable for fish growth performance and do not have any negative impact on growth.

**Conclusion**

From the current study, it can be concluded that stocking density is a critical factor that balances nutrient utilisation in the ecosystem. Therefore, it is recommended to maintain the desirable stocking density in any aquaculture ecosystem for sustainability. The current study suggests that Reba stocking density may be maintained at 2 lahks per hectare for more recovery of organic carbon in fish, and to obtain a higher growth rate, it may be maintained at 1 lakh per ha. Hence, It could be suggested that Reba fry be stocked at 1 -2 lakhs per hectare to obtain higher growth without affecting the environment.

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