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Pulsed Feeding Regimes for the Rearing of *Labeo rohita* (Hamilton) Fry: A Climate Smart Approach

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ABSTRACT

Background: Climate-Smart Aquaculture (CSAq) is built on three pillars, *i.e.*, sustainably increasing productivity and income, improving resilience and mitigating climate change, respectively. Feed constitutes 60%-80% of the total production cost in aquaculture. The reduction of feed cost as well as the pollution originating from uneaten feed have always attracted the attention of aquaculture researchers. Adoption of an optimal feeding regime reduces both feed cost and nutrient loading in the ponds and in turn, enhances the income of farmers and reduces environmental impacts of aquaculture practices. Mixed feeding schedules, pulsed feeding or intermittent feeding have been proposed as some of the climate-smart methods to reduce feed cost and pollution by aquaculture and it has been successfully tested in many fish species. There is lack of sufficient reports on the impact of feeding regimes on the rearing of *Labeo rohita*, Rohu fry. The information will be useful to farmers for rearing of fry to fingerlings with reduced cost and environmental impact.

Methods: A 90-day experiment was conducted to evaluate the growth performance and feed utilization of *Labeo rohita* fry under pulsed feeding regimes as a climate smart approach. For the experiment, the feeding regimes followed were, control (daily feeding @5% of the biomass), 1D4R (1 day starvation+ 4 days refeeding at 5% of the biomass), 2D12R (2 days starvation+ 12 days refeeding at 5% Biomass), 4D16R (4 days starvation+ 16 days refeeding at 5% biomass). The data obtained were subjected to statistical analysis using SAS 9.3. PROC GLM was used for analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test was used for all possible pair comparison of treatments at 5% level of significance.

Result: The fishes under 1D4R exhibited significantly higher weight gain, SGR and feed conversion efficiency (FCE) and significantly lower FCR and production cost than the control group. Water quality parameters like Total NO_2, NO_3, total ammoniacal nitrogen and total phosphate were higher in the control group than in the treatments. The results suggest that one-day feed restriction followed by four days of refeeding could be an efficient strategy for rearing *Labeo rohita* fry to reduce production costs and environmental impact without compromising growth.

Key words: Economic returns, Environmental impact, Feeding management, Fish fry.

INTRODUCTION

Fisheries, sunrise sector of the Indian economy, contributes to the nutrition, income and employment of millions of people in the country. The vulnerability of fisheries sector to climate change has increased over time. Phenomena like accelerated sea level rise and increased frequency of extreme events such as flooding in coastal areas, cyclones and storm surges in the low-lying deltaic regions have been specifically emphasized by the Intergovernmental Panel on Climate Change (IPCC) (IPCC, 2014). Under these changing environmental conditions, the focus need to be shifted to the popularization and adoption of climate-smart aquaculture strategies to improve the nutritional and livelihood security of the growing population. Climate Smart Aquaculture (CSAq) is built on three pillars: sustainably increasing productivity and income (Pillar 1), improving adaptation and resilience to climate change (Pillar 2) and reducing and/or removing GHG emissions (Pillar 3) (FAO, 2013). India is the 4th largest producer of farmed fish in the world. But it is also a known fact that aquaculture produces waste that may harm the environment. When fishes are fed, organic wastes are produced in the form of particulate (mainly uneaten food and feaces) and soluble substances (excreta), which increase the biochemical oxygen demand and concentration of dissolved nitrates and phosphates. Only a portion of the feed that is given is utilized in fish production. Rest of the unused feed can cause high nutrient buildup in the pond leading to eutrophication and algal bloom. One of the most important factors determining the entry of excess nutrients into the aquatic environment is the feeding strategy or feeding

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regime. An aquafarmer can improve profitability by adopting an optimum feeding regime. Although the recommended feed rations are based upon extensive research on fish nutrition, farmed fish show marked variation in appetite both within and between days, which are yet to be studied in detail. Hence understanding the optimum feeding regime will help farmer to evade loss by preventing overfeeding or underfeeding (Noble *et al.,* 2007). This in turn will improve the feeding efficiency, decrease competition and reduce feed wastage resulting in reduced aquatic pollution (Bureau *et al.,* 2006).

Mixed feeding schedules, pulsed feeding or intermittent feeding have been proposed as some of the CSAq methods to reduce feed costs and pollution by aquaculture, in the face of changing climatic conditions and have been successfully tested in many fish species (Pankaj *et al.,* 2017) including Nile tilapia (*Oreochromis niloticus*) (Bolivar *et al.,* 2006; Opiyo *et al.,* 2014), Gilthead sea bream (*Sparus aurata*) (Yigit *et al.,* 2012); two band Seabream (*Diplodus vulgaris*) (Bulut *et al.,* 2014) Asian Seabass (*Lates calcarifer*) (Azodi *et al.,* 2016) Atlantic cod (Bjornevik *et al.,* 2021) *etc*. Several studies have been reported on the effect of feeding regimes on growth and production economics in carp species like common carp (Sultana *et al.,* 2001), gold fish (Belsare and Dhaker, 2019), grass carp (Gou *et al.,* 2023) and *Catla catla* (Mukherjee and Maitra, 2015).

Good quality fish seed is one the major prerequisites for successful aquaculture production. Captive rearing of fish seed *i.e.* rearing of early stages (spawn to fry and fry to fingerling stages) through appropriate feed management in nursery pond is being demonstrated as a climate resilient practice in flood prone areas of India (Prasad *et al.,* 2014). It is also a sustainable livelihood option for rural farmers. Irrespective of the phases, nursery, rearing or grow-out, feed is one of the most expensive component in fresh water aquaculture. Adoption of optimal feeding regimes in all the phases of aquaculture will not only help farmer to increase his profits but will also contribute to climate action through reduced environmental pollution. Reports on the effect of pulsed or intermittent feeding on growth production and aquatic environmental parameters are scanty for fish fry. *Labeo rohita* (Hamilton) (commonly known as rohu), an Indian major carp, is one of the most preferred species in the Indian subcontinent, which contributes to more than 60% of the total carp production in India (Mohanta *et al.,* 2008). The present study focuses on the effect of pulsed feeding regimes on the water quality, growth performance, feed intake and feed efficiency during the rearing of *L. rohita* fry. The study will be useful to farmers for adoption of the practice as a climate smart one with reduced cost and environmental impact.

MATERIALS AND METHODS

Experimental fish and feeding trial

The experiment was carried out in rectangular outdoor concrete tanks of the research facility belonging to the ICAR-Central Institute for Women in Agriculture, Bhubaneswar (Latitute 20.2920615° and Longitude 85.7769064°) for a period of 90 days from September 2023 to November 2023. Twelve tanks were used, each of 2 m^2 area. Before the experiment, all tanks were drained completely and were exposed to sun's rays for 7 days till the tanks were completely dried. Tanks were then refilled maintaining water level at 0.75 m depth throughout the experimental period of 90 days. The tanks received neither supplemental aeration nor fertilization. At the beginning of experiment, twelve tanks were stocked with fry of *Labeo rohita* averaging 0.39±0.007 g, at a density of 30 fishes per tank after acclimatization in triplicate following a completely randomized design. Extruded floating feed (containing 26% CP, 4% crude fat, 6% fibre and 11% moisture (Fishwell, Anmol Feeds, West Bengal, India) were crushed to powder form and fed in two meals daily (09 00 and 15 00 hrs) by broadcasting on the water surface. The fishes were fed @5% body weight in four feeding regimes, *i.e.* daily feeding (C), 1 day starvation + 4 days refeeding (1D4R), 2 days starvation + 12 days refeeding (2D12R)) and 4 days starvation + 16 days refeeding (4D16R) to assess the impact of the pulsed feeding on the survival, growth, FCR, FCE, PER and production cost. The quantity of feed was adjusted every fifteenth day according to the biomass, when the weight and length measurements were recorded. At the end of the experiment, all fish were harvested and individually weighed to obtain total fish yield per feeding schedule. The effect of the different feeding regimes on the water quality was also studied. Water quality parameters notably alkalinity, pH and temperature were measured every 2 weeks. Water temperature was measured insitu, while pH was measured using PHTest 10, Eutech instruments. Water samples were brought to the laboratory for estimation of dissolved oxygen (Winkler's method), total alkalinity, total hardness and inorganic nutrients, *viz,* total ammoniacal nitrogen (TAN), nitrite, nitrate and phosphate, following standard methods (APHA, 2015). For survival rate, dead fish were removed, counted and recorded. Water exchanges every two weeks interval at 75% of water volume were used to maintain water quality.

The experiment as described here did not include procedures that exposed the fish to pain. The fish were also not exposed to stress beyond what can normally occur in a farming situation. The experiment therefore did not require permission from an experimental animal committee at the time. The fish were starved for 24 h prior to weighing.

Bio-growth parameters and production costs

The survival, growth performance and feed utilization characteristics were calculated as follows:

$$\text{Survival (\%)} = \frac{\text{Final fish number}}{\text{Initial fish number}} \times 100$$

$$\text{Weight gain (WG, g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Specific growth rate (SGR), \% day-1} = 100 \times \frac{(\ln W_t - \ln W_0)}{(t - t_0)}$$

Where,

Wt = Mean weight (g) at day t, W_0- Mean weight at day t_0

Length gain (LG, cm) =

Final length (cm) - Initial length (cm)

$$\text{Condition factor (CF, g cm}^{-1}\text{)} = \frac{\text{Live body weight (g)}}{\text{Total body length (cm}^3\text{)}} \times 100$$

$$\text{Food conversion ratio, (FCR)} = \frac{\text{Dry feed given (g)}}{\text{Wet weight gain (g)}}$$

$$\text{Food conversion efficiency, (FCE)} = \frac{\text{Wet weight gain (g)}}{\text{Dry feed given (g)}}$$

$$\text{Protein efficiency ratio, (PER)} = \frac{\text{Wet weight gain (g)}}{\text{Crude protein in feed}}$$

$$\text{Survival (\%)} = \frac{\text{Total number of animals harvested}}{\text{Total number of stocked animals}} \times 100$$

$$\text{Compensation coefficient (CC)} = \frac{\Delta T}{\Delta C}$$

Where,

$$\Delta T = \frac{\text{Average WG in the treatment group (g)}}{\text{Number of feeding days (day)}}$$

$$\Delta C = \frac{\text{Average WG in the control group (g)}}{\text{Number of feeding days (day)}}$$

Production cost =

$$\frac{\text{Feed consumed per fish in Kg} \times \text{Feed cost/kg}}{\text{Final weight of fish in kg}}$$

Statistical analysis

The data obtained through the experiments on growth, feed intake and feed efficiency of *Labe rohita* fry were subjected to statistical analysis using SAS 9.3. PROC GLM for analysis of variance (ANOVA) and Tukey's Honestly significant difference (HSD) test were used for all possible pair comparison of treatments at 5% level of significance.

RESULTS AND DISCUSSION

The physicochemical parameters of water quality of the four different treatments (Control, 1D4R, 2D12R and 4D16R) analyzed during the experimental period are shown in Fig 1. Different nutrient levels in the treatment tanks during the culture period are depicted in Fig 2. Temperature was in the range of 26.5-34.8°C during the rearing period. Dissolved oxygen was in the range of 3.83 to 4.89 mg/l and it increased during the experimental period. There was no marked difference in the pH of water, as no marked difference was observed in the value of free CO_2. The value of total alkalinity and hardness of water did not show any conspicuous variation between the treatments. Total NO_2, NO_3, Total ammoniacal nitrogen and total phosphate were higher in the control group than in the treatments.

Survival (%) was recorded to be highest among the control group of *Labeo rohita* fry and the least in the group 4D16R (Table 1). The mean survival rates obtained were 96.67% for the control group followed by 1D4R, 2D12R and 4D16R. However, the apparent difference in survival (%) was not statistically significant (P>0.05) between the different treatments. Body weight gain and SGR were significantly higher (P<0.05) in the treatment group 1D4R than the control and other treatment groups (Table 1).

Increased proportion of body weight to length as indicated by CF was observed in 1D4R followed by 4D16R, Control, 2D12R. Feed consumption was significantly high (P<0.05) in 1D4R group followed by Control, 2D12R and 4D16R. *Labeo rohita* fry under the feeding regimen of 1D4R had significantly lower FCR (P<0.05) among the treatment groups although the FCR did not significantly vary between the control, 2D12R and 4D16R (Table 2). 1D4R group had the highest feed conversion efficiency. The production cost was the least in 4D16R group of fishes but it was not significantly different between 1D4R, 2D12R and 4D16R (P>0.05).

During the experimental period, DO concentrations, temperature and pH values were within the acceptable range as recommended for the Indian major carps fry rearing (Paul *et al.,* 2017). The different starvation and refeeding regimes did not result in a conspicuous difference in the water quality parameters between the treatments (Fig 1 and 2). This may be because of comparatively long refeeding period followed in the treatments resulting in water quality as comparable to that of control. This aligns with the result reported by Guevara *et al.* (2018) who reported that water quality was not affected by intermittent feeding on juvenile longfin yellowtail *Seriola rivoliana* (Valencinnes, 1833). None the less, a positive effect on the water quality by the increased length of starvation in amur catfish (*Silurus asotus*) fingerlings has been reported by Holeh *et al.* (2020). In the present study, the different feeding regimes might have significantly affected the water quality parameters if the growing period of the fish was longer. Moreover, the regular water exchange and water quality monitoring might have prevented the visible difference in water quality parameters between the treatments.

Feeding strategies for a short or long term can be a tool to overcome many problems such as deterioration of water quality, increasing feed prices and disease outbreaks. Several studies have reported that optimized feeding regimes such as feeding time and feeding frequency during the production cycle can reduce the cost of feeding without slowing growth thereby enhancing profit (Nicieza and Alvarez, 2009 and Abdel Aziz, 2016). Many fish species have demonstrated the capacity to withstand short or long duration starvation (Secor and Carey, 2016). This can be attributed to the compensatory growth which is happening after starvation intervals. There are a group of factors that control compensatory growth such as the response of fish species to re-feeding, the duration of feeding restriction, fish size and age, rearing practices and dietary protein (Hayward and Wang, 2001).

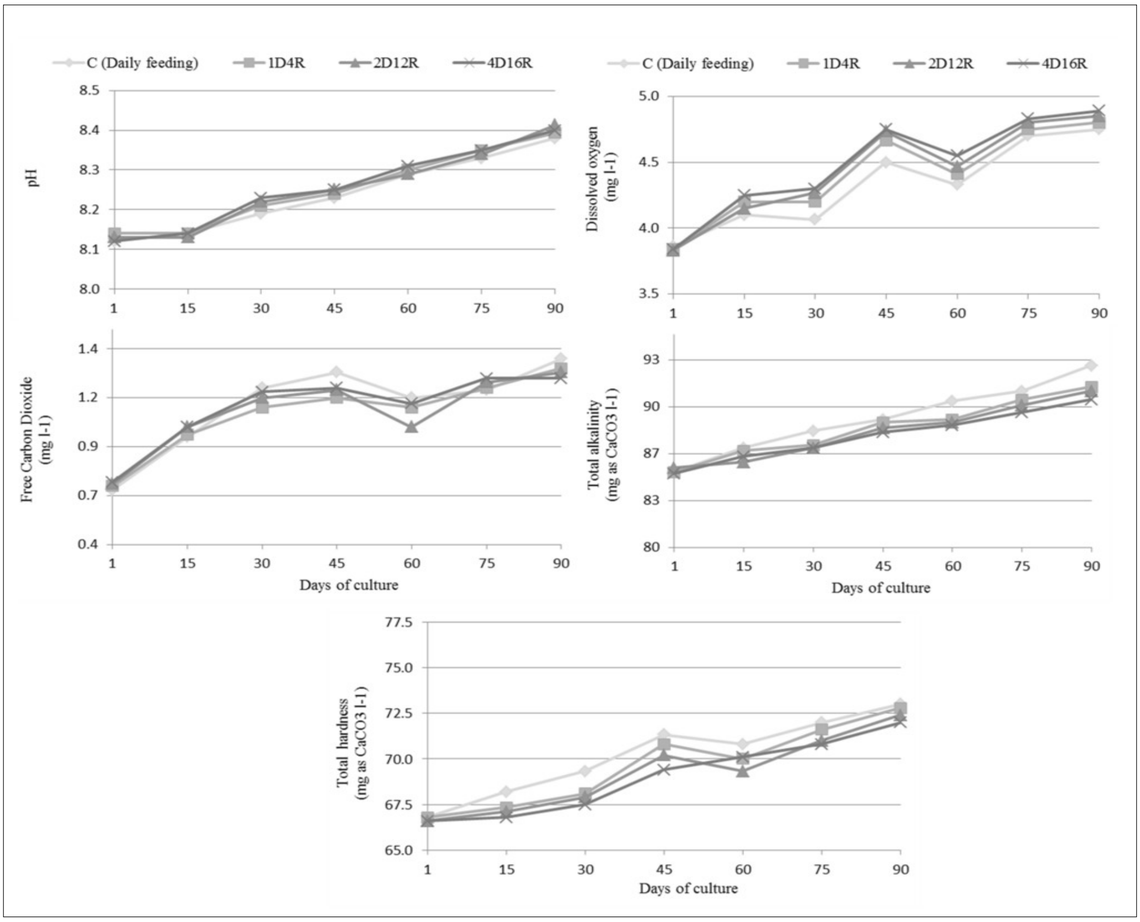

Fig 1: Water quality parameters in the tanks during the rearing of *L. rohita* fry.

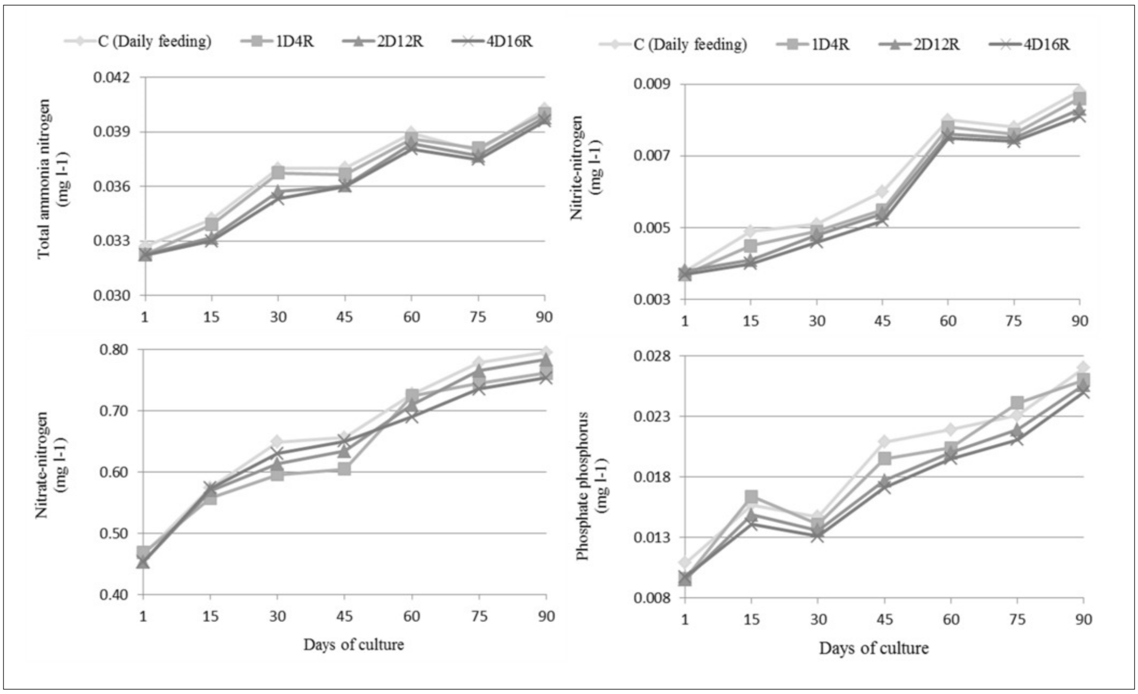

Fig 2: Different nutrient levels in tank water during the rearing of *L. rohita fry* (n=3).

At the end of the experiment, the *Labe rohita* fry fed under the shorter starvation feeding regime of 1D4R were able to compensate fully as indicated by the compensation co-efficient whereas the other treatment groups exhibited only partial growth compensation (Table 1). As per Jafari *et al.* (2018), short-term starvation can lead to fast growth and greater feed efficiency in juvenile fish thereby reducing feed costs. Abdel Aziz (2024) has also reported better feed conversion efficiency and other physiological factors in red hybrid tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*) fingerlings subjected to short-term starvation. Also, in Nile tilapia *Oreochromis niloticus* fingerlings better or similar growth was found when fed every other day (Bolivar *et al.,* 2006; El-Araby *et al.,* 2020). Likewise, Bjornevik *et al.* (2017) reported that alternate-day feeding resulted in 13% more weight gain, lower FCR and production in Atlantic Cod (*Gadus morhua*) juveniles. Body weight gain, length gain and SGR were higher in the treatment group receiving feed under 1D4R feeding regime (Fig 3). The higher SGR in the 1D4R group of fish also suggests overcompensation (Table 1). This may be because of the reason that during short-term starvation, the intestinal feeding efficiency is naturally increased, which results in a more effective absorption of nutrients following re-feeding. Tian *et al.* (2010) and Xiao *et al.* (2013) have also proved that short-term fasting results in complete compensatory growth of fish juveniles accompanied with hyperphagia, or an increase in the fish's appetite, which leads to improved feed efficiency. Condition Factor which is affected by age, season, sexual maturity, gender and nutritional conditions is used to determine the feeding activity in fish and whether it is best utilizing a source of nutrition (Kop *et al.,* 2019). No significant difference was effected in the CF with the different feeding regimes tested although highest CF was observed in 1D4R followed by 4D16R, Control and 2D12R (Table 1).

Reports on better growth performance during longer periods of starvation followed by refeeding are also available. For example, starvation followed by refeeding was reported to improve growth parameters in tinfoil barb (*Barbonymus schwanenfeldii*) wherein four days of starvation followed by 12 days re-feeding regime showed better growth parameters than fish fed daily (Eslamloo *et al.,* 2012). In the present study longer starvation period did not yield a better result in terms of growth, feed intake and feed efficiency (Table 1 and 2). On the contrary, Arguello-Guevara *et al.* (2018) have reported a non-significant effect of two days of fasting- one-day feeding on the growth, feed intake, feed efficiency and morphological indices of juvenile longfin yellowtail *Seriola ravoliana*.

The partial growth compensation in the 2D12R and 4D16R group of fishes (Table 1) might be because of the reason that feed deprivation for longer periods might have caused the degradation of endogenous sources of energy to maintain the fish's physiological homeostasis leading to decrease in weight gain accordingly (Zheng *et al.,* 2016). Yengkokpam *et al.* (2013) also reported a negative effect of longer period of starvation than two days per week on *L. rohita* fingerlings. Similar results have been reported also by Gou *et al.* (2023). Xavier *et al.* (2023) have also reported a partial compensation of growth in silver pompano juveniles (*Trachinotus mookalee*) under the feeding regimen of 2S5RF when compared to juveniles fed daily.

Table 1: Effect of different feeding regimes on growth performance indices of *Labeo rohita* fry for 90 days.

Parameters	Treatments			
	C	1D4R	2D12R	4D16R
Initial weight (g)	0.39±0.01	0.39±0.01	0.38±0.02	0.40±0.02
Final weight (g)	2.13±0.15	3.92±0.17	1.69±0.28	1.60±0.12
SGR (%/day)	1.71±017^{b}	2.32±0.16^{a}	1.6±0.07^{b}	1.42±0.11^{b}
CF (g cm^{-1})	0.78±0.15^{a}	0.89±0.17^{a}	0.75±0.21^{a}	0.87±0.33^{a}
Weight gain (%)	368.24±73.25^{b}	714.57±112.21^{a}	324.06±27.30^{b}	133.23±10.57^{b}
Length gain (%)	79.54±7.63^{b}	103.03±10.17^{a}	72.53±9.49^{b}	56.92±12.68^{b}
Survival %	96.67±3.33^{a}	94.45±6.94^{a}	93.33±8.82^{a}	87.78±12.62^{a}
Compensation co-efficient		1.934004	0.860909	0.717231

The value represented by the same superscripts are at par with each other (P>0.05).

Table 2: Effect of different feeding regimes on feed utilization and production cost of *Labeo rohita* fry.

Parameters	Treatments			
	C	1D4R	2D12R	4D16R
Feed intake/fish (g)	5.29±0.37^{b}	7.42±0.37^{a}	3.6±0.14^{c}	3.04±0.03^{c}
FCR	3.71±0.38^{a}	2.69±0.31^{b}	2.91±0.19^{ab}	2.98±0.41^{ab}
FCE (%)	27.16±2.85^{b}	37.54±4.16^{a}	34.46±2.37^{ab}	34.04±4.76^{ab}
Production cost	217.14±13.34^{a}	176.16±16.61^{b}	166.55±8.31^{b}	160.12±15.87^{b}

The value represented by the same superscripts are at par with each other (p>0.05).

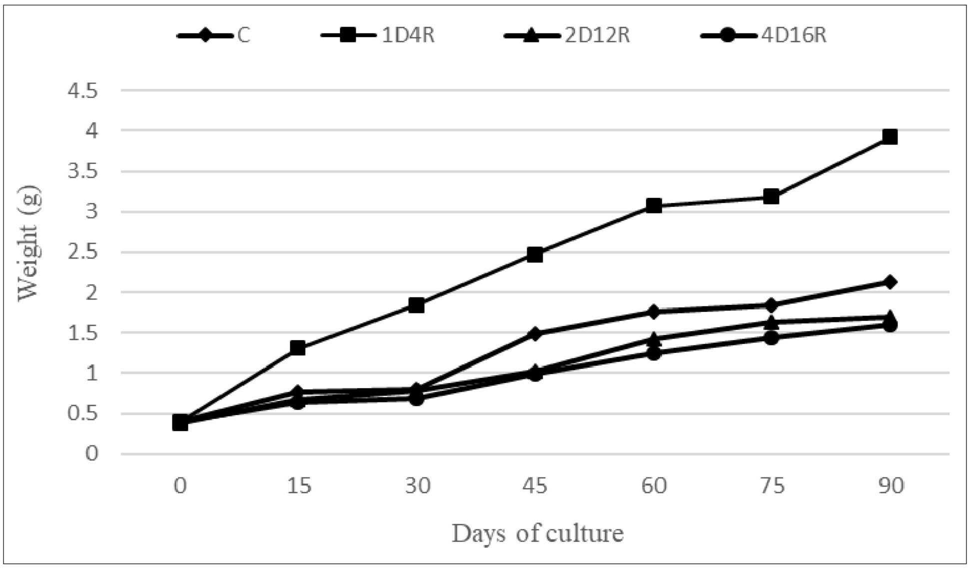

Fig 3: Growth pattern (weight) of *L. rohita* fry during the rearing period.

Feed conversion ratio (FCR) of the treatment groups were lower than the control with the 1D4R treatment group exhibiting significant difference ($P<0.05$) (Table 2). Starvation and re-feeding regimes have documented an improved feed conversion ratio (FCR) in several species of fish (Foss *et al.,* 2009; Heide *et al.,* 2006; Skalski *et al.,* 2005). The production cost decreased significantly with increase in length of starvation (Table 2) without adversely affecting the SGR. Bjornevik *et al.* (2021) demonstrated that feeding costs can be drastically reduced without compromising biomass growth by adopting feeding on alternate days during the on-growing period of Atlantic cod. At the end of the experiment, when total feed intake was measured the deprived groups consumed significantly less food than the control group except for the tratemnet 1D4R which showed the highest total feed intake (Table 2). But the fish with 1D4R feeding regime utilized the feed better as the FCR was lower compared to the control group (Table 2). As feed normally makes upto 50-70% of the production cost, a lower FCR is crucial from an economic perspective (Rana *et al.,* 2009). The 1D4R group fishes had the lowest FCR and lower production cost than the control group making it a better feeding regime when compared to the control, 2D12R and 4D16R that can be followed by farmers for the rearing of rohu (*Labeo rohita*) fry.

CONCLUSION

As conclusion, following a feeding regime of one day starvation followed by four days refeeding can be adopted as a climate smart practice, not only to improve the adaptation or resilience of farmers to climate change by reducing the production cost but also to reduce the environmental impact caused due to water pollution through the particulate or soluble matter generated from the uneaten feed or excessive feed. Further research on the effect of the pulsed feeding strategy on the grow out farming of *L.rohita* will provide an insight into the feed management strategy that can be followed to minimize not only the production cost but also the negative environmental impact caused by the leftover or uneaten feed.

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Conflict of interest

All authors declared that there is no conflict of interest.

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