

# Growth performance and feed utilization of grey mullet fingerlings fed with varying levels of protein during nursery rearing phase

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## Abstract

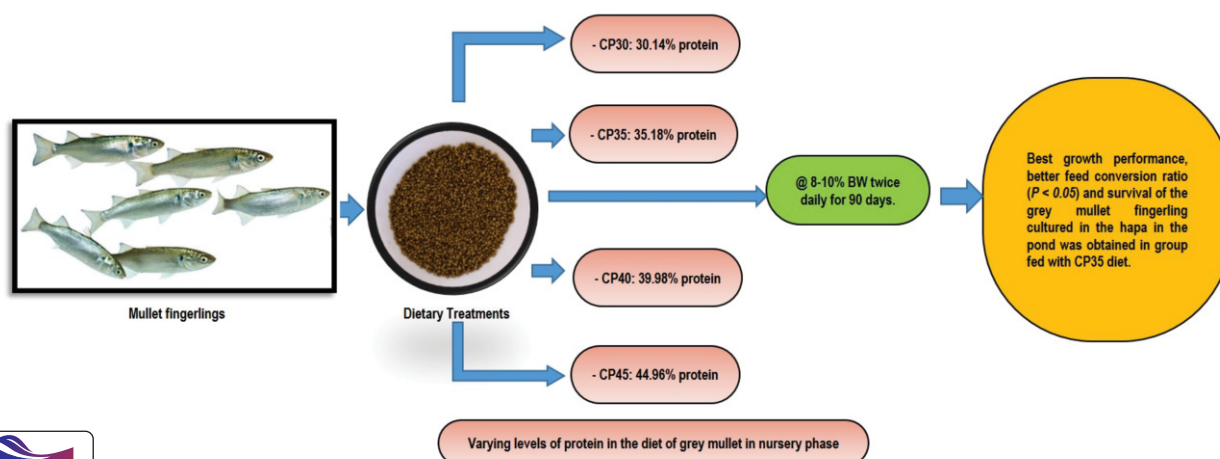
**Aim:** This study evaluates the growth performance and feed utilization of wild grey mullet fingerlings fed with varying levels of crude protein during the nursery rearing phase.

**Methodology:** Grey mullet (*Mugil cephalus*) fingerlings were collected from the wild and were acclimatized under laboratory conditions before the start of the experiment. The fingerlings (initial average body weight of  $2.82 \pm 0.1$  g) were stocked at 200 individuals per hapa (dimension:  $2 \times 2 \times 1$  m) in a brackishwater pond (2500 m<sup>3</sup>) and the experiment was conducted for 90 days in a completely randomized design with three replicates per treatment. Four experimental diets with graded crude protein (CP) levels of 30% (CP30), 35% (CP35), 40% (CP40), and 45% (CP45) on a dry matter basis were formulated using locally available feed ingredients and the effect of different levels of protein on growth performance was assessed.

**Results:** At the end of 90 days of feeding experiment, the fish group fed with CP35 diet showed the best FCR ( $p < 0.05$ ) and recorded the highest growth performance among different treatment groups. Increasing the protein content beyond 35% in the diet revealed no beneficial effect on various growth performance parameters.

**Interpretation:** This study recommends the formulated diet with 35% crude protein for improved growth and survival of grey mullet fingerlings for farming in the pond based hapa system.

**Key words:** Fingerlings, Grey mullet, Growth, Hapa, Protein



## Introduction

Grey mullet is an ideal candidate species for sustainable aquaculture and is gaining popularity among brackishwater farmers due to its position at a lower trophic level in the food chain. Its superior flavor and texture have led to high consumer demand for the fish (Lupatsch *et al.*, 2003). It is gaining momentum among the farming community in India after the successful breakthrough of captive breeding and larval rearing at ICAR-CIBA (Mathew *et al.*, 1999; Sukumaran *et al.*, 2021; Jena *et al.*, 2022). The majority of global grey mullet production is derived from capture fisheries, amounting to approximately 291,000 tons (FAO, 2022). Mullet is an omnivorous fish (Whitfield *et al.*, 2012), ecologically significant species, which makes them suitable for monoculture and polyculture systems (Abdel-Hakim *et al.*, 2001; Mondal *et al.*, 2015). Grey mullet or flathead grey mullet, is scientifically known as *Mugil cephalus*, belongs to family Mugilidae. It is one of the commercially important brackishwater finfish species abundantly available in India (Biswas *et al.*, 2012; Jana *et al.*, 2004). It fetches a good price in the local market due to high consumer preference (De *et al.*, 2012).

*Mugil cephalus* is known for its diurnal and opportunistic feeding behaviour, with the tendency to feed almost continuously throughout the day (Jamabo *et al.*, 2015; Mondal *et al.*, 2015). In India, mullet seeds are harvested along the coastal water bodies especially in lagoons, adjacent brackish-water lakes, and estuaries (Kailasam *et al.*, 2019). Considering the suitability of this species for farming in brackishwater ponds, researchers have been encouraged to develop techniques on captive breeding and seed production of this species. (Mathew *et al.*, 1999; Sukumaran *et al.*, 2021). Grey mullet farming in India follows a traditional aquaculture approach, relying on naturally sourced fingerlings collected from the wild. Consequently, its cultivation is primarily centered in regions close to areas abundant with wild seeds. Further, the scientific information regarding their nutrient requirement and feed utilization is meagre (García-Márquez *et al.*, 2021). The foremost critical factor in captive finfish aquaculture is the availability of appropriate cost effective feed, which typically constitutes approximately half of the total farming expenses (Assan *et al.*, 2021).

Feed quality at early stages is vital for fast growth and improved survival of fishes. Protein requirements tend to be lower for herbivorous and omnivorous fish species. However, fish require more protein during their early life stages, which decreases as they grow older (Craig *et al.*, 2017). Fish farming success depends on the protein needs of each species and life stage, as well as the feed nutrient cost, particularly protein, which is the most costly nutrient of formulated diets for fish (Altunok *et al.*, 2017; El-Tawil *et al.*, 2014). Developing a cost-effective, nutritionally balanced feed depends on understanding the species' nutritional needs and feed usage (Hardy *et al.*, 2003). As a result, research efforts on mullet feeding has mainly concentrated on investigating alternate protein sources and their impact on the development and feed intake of *Mugil* species

(Altunok *et al.*, 2017; De Carvalho *et al.*, 2010). Previous research findings (De *et al.*, 2012; Koven *et al.*, 2020; Luzzana *et al.*, 2005; Wassef *et al.*, 2001) have suggested around 28-35% dietary crude protein level as optimum for the growth of *Mugil* species under laboratory conditions. However, documented information on the use of formulated feed with varying protein levels on the grey mullet growth under pond conditions is limited, therefore, the current study was conducted to evaluate the optimum protein requirement of grey mullet fingerlings in nursery rearing in hapa in the pond.

## Materials and Methods

**Study site:** The study was conducted from February to May 2020 in association with NGRC- ICAR CIBA and the women self-help group members of Matwad village, adhering to all COVID-19 pandemic protocols. The experiment was carried out at the Brackishwater Aquaculture Research and Demonstration Farm, Matwad, NGRC of ICAR-CIBA, in Navsari, Gujarat. It was conducted in a tide-cum-pump-fed brackishwater earthen pond with a capacity of 2500 m<sup>3</sup> and a water depth of 1.5 m.

**Experimental animals:** The wild collected grey mullet seeds were procured from commercial catches (Machilipatnam district, Andhra Pradesh, India) and transported to the experimental site through air cargo. The fish were identified taxonomically, individually selected, and subsequently stocked in a large hapa. The fingerlings were acclimatized to the pond culture conditions for a period of seven days and thereafter, stocked in the experimental hapas. The experiment was conducted in the hapas (2 x 2 x 1 m; 5 and 10 mm mesh) installed in the pond. The fingerlings of grey mullet (*Mugil cephalus*) with initial average body weight of  $2.82 \pm 0.1$  g were randomly stocked in 4 m<sup>2</sup> (dimensions: 2 x 2 x 1 m) at a density of 200 fingerlings per hapa (Ignatius *et al.*, 2017).

**Experimental diets:** Four diets with different protein content (30, 35, 40 and 45%) were formulated using locally available feed ingredients. The ingredients and proximate composition of the experimental diets are provided in Table 1. All the dry and solid feed materials were coarsely powdered in a hammer mill followed by fine grinding in a micro pulveriser. The powdered mash was strained through a 0.3-mm filter. The mixture, including additives, was homogenized in an extruder with 280 ml of water per kilogram of feed. The mixed mash was delivered to the hopper above the extruder feeder and then extruded using a 2 mm diameter die. The material was dried to less than 12% moisture content and kept in airtight containers before use. The extruder feed was crumbled and sieved to get the required sizes as per requirement. The experimental diets were prepared at the feed mill of Central Institute of Brackishwater Aquaculture (CIBA), Chennai Muttukadu experimental station. Experimental feeds were supplied to triplicate groups (CP30, CP35, CP40 and CP45) of grey mullet fingerlings thrice a day (8-9 am, 1-2 pm and 5-6 pm) until apparent satiation.

**Chemical analyses:** The ingredients and experimental diets proximate composition was analyzed according to the standard procedures (AOAC, 2012). The Kjeldahl method (N x 6.25) was

**Table 1:** Ingredient (%) and proximate composition (%) of the experimental diets

Ingredient/diets (g 100g <sup>-1</sup> )	CP30	CP35	CP40	CP45
Fishmeal	25	30	35	40
Soybean meal	12.67	16.5	19.47	23
Wheat grains	18	14.57	11.4	7.87
Maize	18	15	12	9
Broken rice	18	15	12	9
Wheat Gluten	2	3	4.6	6
Sunflower oil	1.35	1.15	0.95	0.75
Fish oil	1.35	1.15	0.95	0.75
Lecithin	0.5	0.5	0.5	0.5
Mineral and Vitamin Mixture	2	2	2	2
Spirulina	1	1	1	1
Vitamin C	0.03	0.03	0.03	0.03
Choline chloride	0.1	0.1	0.1	0.1
Proximate composition of the experimental diet (%)				
Moisture	10.2	10.11	10.21	10.34
Crude Protein	30.14	35.18	39.98	44.96
Ether Extract	7.64	7.72	7.62	7.69
Crude Fiber	3.81	3.87	3.78	3.71
Total Ash	7.23	7.91	8.88	9.94
NFE	40.98	35.21	29.53	23.36

used for the estimation of moisture, crude protein following acid hydrolysis (Kjeltec 2100, FOSS, Tecator, Sweden). Soxhlet system (SOCS, Pelican, India) technique was used for the crude lipid gravimetric extraction with petroleum ether. In a muffle furnace, the total ash was calculated gravimetrically after 6 hr of combustion at 600 °C. Crude fiber was measured gravimetrically following digestion with acid and alkali and mass loss through comminution for 3 hr at 600 °C. Nitrogen free extract (NFE) was calculated by deducting the sum of moisture, crude protein, crude fat, crude fiber and total ash from 100. All the chemical analyses were carried out in triplicate and recorded as dry matter.

**Analysis of water parameters:** The pond water quality parameters were monitored regularly to maintain optimal conditions for fish health and growth throughout the experiment. Ammonia (mg l<sup>-1</sup>) levels were measured weekly using a Hanna ammonia meter, while pH, temperature (°C), salinity (ppt) and dissolved oxygen (mg l<sup>-1</sup>) were recorded daily during early morning hours with a Eutech cyberscan series 600 portable meter.

**Growth performance:** Samples were taken every 15 days to evaluate the growth performance. For sampling, 50 fish from each experimental hapa were randomly collected to measure the length and weight, and the ration was modified based on the biomass. Survival percentage of the fish was recorded during each sampling and at the end of the experiment. The standard formulae were used to calculate the growth parameters such as final weight (g), daily weight gain (g day<sup>-1</sup>), weight gain (WG%), and specific growth rate (SGR%), as well as feed utilization parameters like feed conversion ratio (FCR), feed efficiency (FE%), protein efficiency ratio (PER), and survival (%) (Hardy and

Barrows, 2003; Ridha *et al.*, 2006).

**Statistical analyses:** The growth parameter and survival data were analyzed by One-way ANOVA to evaluate survival data and growth metrics (SPSS version 16). The means of various feed protein level treatments were compared using Tukey's test when a significant treatment effect was noted.

## Results and Discussion

In the present study, four iso-lipidic practical diets were designed to contain varying protein levels and the final composition matched with the expected nutrient levels. The four experimental diets labeled as CP30, CP35, CP40, and CP45 were analyzed and showed dietary protein level of 30.14, 35.18, 39.98 and 44.96%, respectively. The experimental fish readily accepted the diet, regardless of the dietary protein content. This indicated that there was no problem in feed attractability and palatability due to variation in the protein content from 30 to 45%. The details of the pond water quality parameters recorded during the experimental period are given in Table 2. The pond water pH, temperature, salinity, dissolved oxygen and ammonia were observed in the range of 7.8 to 8.3, 24.6 to 31.5°C, 20 to 28 ppt, 5.2 to 6.8 mg l<sup>-1</sup> and 0.004–0.05 mg l<sup>-1</sup>, respectively and found within the optimal range required for aquaculture farming (Boyd, 1998).

In the present study, the weight gain of fish fed with varied levels of protein diet revealed that the group fed with CP35 diet gained significantly higher weight ( $p < 0.05$ ) as compared to other three treatments (Table 3). Further, it is interesting to note that on

**Table 2:** Water quality parameters recorded during the experimental period

Parameters	Range
Temperature (°C)	24.6-31.5
pH	7.8-8.3
Salinity (ppt)	20-28
Dissolved oxygen (mg l <sup>-1</sup> )	5.2-6.8
Ammonia (mg l <sup>-1</sup> )	0.004-0.05

increasing the protein content of the diet beyond 35% did not increase the growth performance in mullet fingerlings. Previous studies conducted on rainbow trout (Ahmed et al., 2020), red swamp crayfish (Lu et al., 2020) showed that the species growth rates are positively correlated with dietary protein levels, however, in this study, 40% and 45% protein diets did not increase the growth in mullet fingerlings. Kim et al. (2004) recommended that protein levels in the diet, lower or higher than the optimum range, tend to decrease the growth or remain unaffected in juvenile Korean rockfish, because there may be a decrease in the efficiency of protein conversion as some protein is catabolized and protein synthesis is not encouraged. Furthermore, if the diet is protein rich, then a lack of non-protein energy in the feed can result in poor growth performance due to protein preferred catabolism (Winfree et al., 1981).

The type of production system (raceway, cage, open ponds, etc.) can also influence the optimal protein requirement for a fish species (Craig et al., 2017; Izquierdo et al., 2000). Nutrient requirement of fish in pond ecosystem may vary from the controlled condition as fish in open water pond are exposed to several environmental stressors (Robaina et al., 2000). It is important to note that earlier grey mullet dietary protein levels experiments were conducted in indoor settings like aquariums, tubs and tanks (Altunok et al., 2017; De et al., 2012; Talukdar et al., 2020; Yones et al., 2019) whereas the present study was conducted in hapa installed in the pond. The open pond

conditions also have availability of plankton and also contribute toward meeting the nutrient requirement of the culture fishes, which can improve the growth in a filter feeder species. At the end of the study, 35% dietary protein levels increased both final mean weight (g) and specific growth rate (SGR%). However, these parameters showed a reducing trend when protein levels exceeded 35%. In a previous study, the growth rates of *M. cephalus* reared in tank (De et al., 2012), *C. labrosus* in sea cages (Altunok et al., 2017) and *C. chanos* in brackishwater pond (Hussain et al., 2021) were observed from 0.1 to 0.3 g day<sup>-1</sup> when fed with 30% crude protein diet. The limited growth of grey mullet on a diet containing 45% crude protein indicated that providing excess protein more than required may not be of additional benefit. Stagnation of growth or reduction above optimal protein levels has been observed in mugil species, Nile tilapia and silver pomfret (Abdel-Tawwab et al., 2010; Arshad Hossain et al., 2010).

In a previous study, the growth rates of *M. cephalus* reared in tank (De et al., 2012), *C. labrosus* in sea cages (Altunok et al., 2017) and *C. chanos* in brackishwater pond (Hussain et al., 2021) were observed from 0.1 to 0.3 g day<sup>-1</sup> when fed with 30% crude protein diet. Similarly in this study, 0.36 g day<sup>-1</sup> growth rate was observed in *M. cephalus* fingerlings fed with CP30 diet, further, significantly ( $p < 0.05$ ) higher growth rate of 0.46 g day<sup>-1</sup> was found in *M. cephalus* fingerlings fed with CP35 diet. Fortnightly weight gain (g) of grey mullet fingerlings fed with varied crude protein diets for 90 days indicated that the diet containing 35% crude protein had the highest gains when compared to other diets as shown in Fig. 1.

Result demonstrated that fish fed a diet with 35% crude protein showed a significantly better ( $p < 0.05$ ) Feed conversion ratio (FCR -2.21) compared to other dietary treatment. Similarly, Yones et al. (2019) reported that diet containing 35% protein showed better FCR (2.25). The FCR was notably better as compared to previous reports for *Mugil* species (Barman et al., 2005; De et al., 2012; Papapaskeva-Papoutsoglou et al.,

**Table 3:** Growth performance, feed utilization and survival of grey mullet fingerlings fed with different dietary crude protein feeds for 90 days

Parameters	CP30	CP35	CP40	Cp45
Initial weight (g)*	2.82 ± 0.1	2.82 ± 0.1	2.82 ± 0.1	2.82 ± 0.1
Final weight (g)	34.81 ± 0.66 <sup>a</sup>	44.58 ± 1.18 <sup>b</sup>	40.31 ± 0.60 <sup>c</sup>	39.04 ± 0.56 <sup>d</sup>
Weight gain (g)	31.99 ± 0.62 <sup>a</sup>	41.76 ± 1.21 <sup>b</sup>	37.50 ± 0.65 <sup>c</sup>	36.22 ± 0.78 <sup>d</sup>
DWG (g day <sup>-1</sup> )	0.36 ± 0.01 <sup>a</sup>	0.46 ± 0.01 <sup>b</sup>	0.42 ± 0.01 <sup>c</sup>	0.40 ± 0.01 <sup>c</sup>
WG (%)	1134.5 ± 4.83 <sup>a</sup>	1481 ± 4.28 <sup>b</sup>	1330 ± 12.5 <sup>c</sup>	1284.5 ± 10.03 <sup>d</sup>
SGR (%/day)	2.80 ± 0.13 <sup>a</sup>	3.08 ± 0.14 <sup>b</sup>	2.96 ± 0.14 <sup>c</sup>	2.93 ± 0.15 <sup>c</sup>
FCR	2.71 ± 0.02 <sup>a</sup>	2.21 ± 0.08 <sup>b</sup>	2.63 ± 0.02 <sup>a</sup>	2.65 ± 0.06 <sup>a</sup>
FE	0.62 ± 0.003 <sup>a</sup>	0.70 ± 0.003 <sup>b</sup>	0.59 ± 0.003 <sup>c</sup>	0.63 ± 0.003 <sup>a</sup>
PER	1.23 ± 0.01 <sup>a</sup>	1.28 ± 0.02 <sup>b</sup>	0.95 ± 0.01 <sup>c</sup>	0.84 ± 0.01 <sup>d</sup>
Survival (%)*	100	100	100	100

Feed utilisation values are expressed as mean ± SD of three replicates per treatment (n=3), and values with different superscript letters are significantly different ( $p < 0.05$ ) among treatments and values with \* are non significantly ( $p > 0.05$ ) different among treatments. DWG: daily weight gain; WG: weight gain; FCR: Feed conversion ratio; FE: Feed efficiency; PER: Protein efficiency ratio

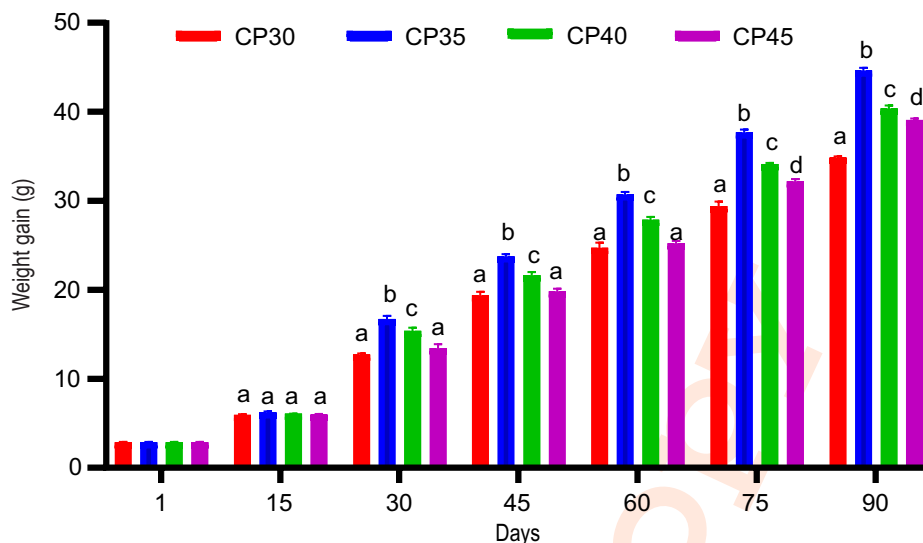


Fig. 1: Fortnightly weight gain (g) of grey mullet fingerlings fed with different crude protein diets for a period of 90 days.

1986). Interestingly, a few studies have reported higher FCR when fish were fed lower dietary protein levels probably due to insufficient nutrient levels to support the optimal growth (Yones et al., 2019; Zhou et al., 2007). In general, fish regulate their feed intake in order to meet their metabolic energy needs (Kim et al., 2000). However, fish typically increase their feed intake to compensate for nutrient deficiencies when fed with decreased protein levels (Sa et al., 2014). In this study, it was observed that the feed efficiency was maximum when the fish were fed with a diet containing 35% crude protein. Similar trend was also observed in previous studies of different crude protein diet on number of fish species, including grey mullet (De et al., 2012), thick lip grey mullet (Altunok et al., 2017) and other species such as milkfish (Hussain et al., 2021), tire track eel (Loan et al., 2025). Fish utilize dietary protein based on the balance of protein and non-protein energy sources. As a result, excess protein in the diet may be catabolized as an energy source (NRC, 2011), or it may inhibit growth due to increased energy requirements. Hence, the present study showed that the higher growth in grey mullet fingerlings may be attributed to efficient feed consumption at ideal dietary protein levels.

The perusal of data showed that the protein efficiency ratio decreased when the dietary protein content was more than 35%. Protein efficiency ( $1.28 \pm 0.02$ ) was significantly ( $p < 0.05$ ) best in 35% protein diet. Further, increase in the level of protein to 40% ( $0.95 \pm 0.01$ ) and 45% ( $0.84 \pm 0.01$ ) resulted in lower PER. Similarly, diet containing 300 g protein in 1 kg feed showed higher level of PER ( $1.95 \pm 0.07$ ). In addition, an increase in the level of protein in the diet to 320g ( $1.87 \pm 0.04$ ) and 340 g ( $1.59 \pm 0.02$ ) showed a reduction in PER level (Talukdar et al., 2020). The results of this study revealed that the protein utilization decreased in fish beyond a dietary protein level of 35%, indicating that some protein was being used as a catabolic substrate. This, in turn, led

to an expected reduction in protein accretion, a phenomenon observed in mugil species (Altunok et al., 2017; De Carvalho et al., 2010; De et al., 2012; Talukdar et al., 2020; Yones et al., 2019), Nile tilapia (Abdel-Tawwab et al., 2010), rohu (Gangadhara et al., 1997) and silver barb (Mohanta et al., 2008). Our findings are consistent with the previous reports indicating that dietary protein levels ranging between 28-35% promote optimal development and feed consumption in mullet species under laboratory conditions (Altunok et al., 2017; De et al., 2012; Talukdar et al., 2020; Yones et al., 2019).

In all the treatments, 100% survival of *M. cephalus* was recorded over the experimental period. Prior studies on the effect of different dietary protein levels on *M. cephalus* (Talukdar et al., 2020), *Cutler mongolicus* (Qian et al., 2022), *Sardinella brasiliensis* (Strzelecki et al., 2018) and juvenile bluegill sunfish (Yang et al., 2016) have reported similar survival findings. These findings imply that dietary protein intake may not be a major contributing factor to death. The better survival rate found in this study suggested that the feeding management and environment of the nursery culture of fish were appropriate for obtaining the experimental results. Therefore, increasing protein levels higher than 35% affected fish growth and protein efficiency ratios and diet containing 35% protein was considered optimal for nursery rearing of grey mullet fingerlings in hapa in ponds.

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**Authors' contribution:** Pankaj A. Patil: Conceptualization, Investigation, Validation, Methodology, Visualization, Writing – Original draft, Writing – revision and editing; T. Hussain: Data Curation, Revision and Editing Manuscript; K. Ambasankar: Supervision, Feed preparation and Nutrient analysis; P. Mahalakshmi: Resources supply; M. Kailasam: Supervision.

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**Research content:** The content of this study is original and has not been published elsewhere.

**Ethical approval:** The study was undertaken with the approval of the statutory authorities of the Central Institute of Brackishwater Aquaculture, Chennai, India. Grey mullet are not an endangered fish as per the provisions of the Government of India's Wildlife Protection Act of 1972.

**Conflict of interest:** No conflict of interest was reported by the author(s).

**Data availability:** The data that support the findings of this study are available from the Corresponding author.

**Consent to publish:** All authors agree to publish the paper in *Journal of Environmental Biology*.

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