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Effects of dietary protein levels on growth performance and whole-body composition of juvenile grey mullet (*Mugil cephalus*) reared at 15 ppt salinity

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Abstract

Grey mullets are euryhaline fish with considerable potential for sustainable aquaculture diversification. However, their dietary protein requirements under different rearing conditions remain insufficiently understood. This study investigated the effects of dietary protein levels on growth performance and whole-body composition of juvenile grey mullet (*Mugil cephalus*) reared at a salinity of 15 ppt. A 60-day feeding trial was conducted using juvenile mullets with an initial average body weight of 7.10 ± 0.09 g. A total of 120 fish were randomly distributed among four dietary treatments (CP25, CP30, CP35, and CP40), each with three replicate tanks containing 10 fish. Fish were fed four semi-purified, isocaloric (383 kcal dietary energy/100 g) and isolipidic (60 g lipid/kg) experimental diets formulated with graded levels of crude protein (25%, 30%, 35%, and 40%). Fish were fed three times daily to apparent satiation. The results showed that fish fed the CP35 diet exhibited significantly higher weight gain (WG) and specific growth rate (SGR), as well as a lower feed conversion ratio (FCR), compared with the other dietary treatments ($p < 0.05$). In contrast, the protein efficiency ratio (PER) increased significantly with increasing dietary protein levels ($p < 0.05$). Whole-body protein, lipid, and ash contents also differed significantly among dietary treatments ($p < 0.05$). Overall, the findings indicate that a dietary crude protein level of approximately 35% may support optimal growth performance and feed utilization in juvenile grey mullet reared at 15 ppt salinity. These findings provide useful information for developing nutritionally balanced diets for grey mullet aquaculture under moderate-salinity conditions.

Keywords: Grey mullet; *Mugil cephalus*; Dietary protein; Growth performance; Whole-body composition; Salinity.

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Introduction

Aquaculture has become the fastest-growing food production sector globally and is expected to play a pivotal role in meeting the increasing demand for high-quality animal protein while reducing pressure on capture fisheries (Raushon *et al.,* 2026). Among cultured finfish, the grey mullet (*Mugil cephalus*) has attracted increasing attention because of its rapid growth, broad salinity tolerance, omnivorous feeding habit, excellent flesh quality, and suitability for monoculture, polyculture, and integrated multitrophic aquaculture systems. Its remarkable euryhaline nature enables successful culture in freshwater, brackish water, and marine environments, making it an ideal candidate for sustainable aquaculture diversification, particularly in coastal regions where inland saline water resources remain underutilized (Patil *et al.,* 2025).

Dietary protein is the most important and expensive component of aquafeeds, accounting for approximately 40–60% of total feed costs (Hajlaoui *et al.,* 2025). Protein supplies essential amino acids for tissue synthesis, growth, enzyme production, immune function, and osmoregulatory processes (Mannur *et al.,* 2024). However, inadequate dietary protein restricts growth and feed efficiency, whereas excessive protein increases feed costs, nitrogen excretion, and environmental pollution because surplus amino acids are catabolized for energy rather than retained for growth (Aboelyzed *et al.,* 2024). Therefore, determining the optimum dietary protein requirement is essential for maximizing production efficiency while improving economic and environmental sustainability (Paul *et al.,* 2022).

The protein requirement of *M. cephalus* varies considerably with fish size, dietary energy, feed ingredients, culture conditions, and particularly salinity, which strongly influences energy allocation for osmoregulation. Previous studies have reported optimum dietary crude protein levels ranging from approximately 260 to 400 g/kg for grey mullet and related mugilid species cultured under different environmental conditions, including freshwater, seawater, and varying salinity regimes (El-Dahhar *et al.,* 2014; Yones *et al.,* 2019; Khalafalla *et al.,* 2022; Patil *et al.,* 2025; Randazzo *et al.,* 2026). These variations demonstrate that protein requirements are not fixed but depend on environmental conditions, especially salinity (Zaman and Cho, 2026). Juvenile fish cultured under intermediate salinity often experience increased osmoregulatory energy demand, which may alter protein utilization efficiency and consequently modify dietary protein requirements (Paul *et al.,* 2024).

Despite growing commercial interest in grey mullet culture, information on dietary protein requirements under moderate brackish-water conditions remains limited. To date, no study has specifically determined the optimum dietary protein requirement of juvenile *M. cephalus* reared at 15 ppt salinity under Bangladesh's coastal farming conditions. This represents an important knowledge gap because 15 ppt closely

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reflects the salinity found in many coastal ponds and estuarine aquaculture systems in the northern Bay of Bengal, where grey mullet has considerable commercial expansion potential. Moreover, existing protein requirement estimates generated under different salinity conditions cannot be directly extrapolated because salinity substantially affects metabolism, nutrient utilization, and growth performance (Zaman *et al.,* 2022).

Based on previous nutritional studies in mugilids and other euryhaline fishes, dietary protein levels ranging from 250 to 400 g/kg were selected to encompass suboptimal, adequate, and potentially excessive protein concentrations, thereby enabling precise estimation of the optimum protein requirement through polynomial regression analysis. We hypothesized that growth performance, feed utilization, and whole-body composition of juvenile *M. cephalus* cultured at 15 ppt salinity would improve with increasing dietary protein up to an optimum level, beyond which no further biological benefit would be achieved. The present study therefore provides the first comprehensive evaluation of dietary protein requirements for juvenile grey mullet cultured under 15 ppt saline water conditions in Bangladesh, generating practical nutritional recommendations for cost-effective feed formulation and contributing to the sustainable development of coastal aquaculture.

Materials and methods

Experimental design

The grey mullet (mean weight 7.10±0.09 g, total length 0.67±0.02 cm) was sent to the Marine Fisheries and Technology Station (MFTS), BFRI, Cox's Bazar, Bangladesh, along with an inflatable bag filled with oxygen-enriched water. Fish were housed in a cement reservoir measuring 3 m × 2 m × 1 m, with a capacity of 10,000 L and fully equipped with an adjustable air-exchange system. Using a professional pump, saline water was collected and stored in containers (2 m×1 m×0.5 m, with a 5000 L capacity) and water purification modules (100 μm). The saline water was used in the research facility after 14 days and was deposited into reservoirs with an intake of 500 L (1 m×1 m×0.70 m). For two weeks, the fish were acclimated to a maintenance diet. Following SW administration, the salt content steadily increased by 1 ppt per day until it reached 15 ppt. At that point, the fish recovered from injuries sustained during transportation. After warming up, a completely randomized design (CRD) was used to assign 120 fish with a mean weight of 7.10±0.09 g to each of the four experimental groups: CP_{25}, CP_{30}, CP_{35}, and CP_{40}. Ten fish were kept in each tank (n=12), which measured 93 cm in diameter and 44 cm in height. At 10:00, 15:00, and 18:00 h, the fish were fed according to their respective diets until they were no longer hungry. Every 15 days, the body weight was measured to track progress. The interior of each tank was emptied by funneling before morning feeding, and the saltwater was refilled with the same amount of water that was removed from the storage

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container. At three-day intervals, about 20–25% of the treated water in each tank was replaced with SW at 15 ppt throughout each testing phase. After receiving approval from the BFRI headquarters' research ethics committee in Mymensingh, Bangladesh, all animal experiments were conducted carefully in accordance with accepted standards for animal handling and care.

Composition and administration of formulated diets

Experimental diets CP_{25} (25 g CP/kg), CP_{30} (300 CP/kg), CP_{35} (35 g CP/kg), and CP_{40} (40 g CP/kg) were proposed. Semi-purified iso-nitrogenous (25–40 g/kg dietary crude protein), iso-caloric (about 36 kcal DE/100 g), and iso-lipidic (6 g/kg) were among them. In accordance with the instructions, the coarsely ground materials were measured accurately and placed in sealed containers. The ingredients (aside from oils and additives) were thoroughly mixed with the necessary amounts of water to prepare the dough. After 20 minutes of vaporization in an automatic pressure cooker, the dough was dried at room temperature. The dough was then mixed with oil and the additives (CMC and a blend of vitamins and minerals). An un-extruded single-screw stretching device (Wecoxex, China) was then used to mold and compress the mixture into equivalent granule strands (diameter 1.5 mm). We let them air-dry at a reasonable temperature before browning them at 35-40°C to remove about 10% of the moisture. Subsequently, the dry pellets were cut into 4–6 mm lengths, placed in impermeable polyethylene zipper bags, sealed, labeled with the appropriate dietary category titles, and stored in a freezer at -40°C until needed (Gupta *et al.,* 2021). Fish feed consumption was recorded daily using the uneaten feed criterion (Table 1).

Physico-chemical attributes of water

Ongoing surveillance of the water's physicochemical properties, including pH, temperature, salinity, dissolved oxygen (DO), Nitrate-nitrogen (NO_3-N), Nitrite-nitrogen (NO_2-N), and Alkalinity, was conducted throughout the investigation. A digital multi-parameter analyzer (Hach, USA) was installed to quantify salinity, temperature, DO, and pH. Normative regulations of APHA (2023) were applied to figure out additional parameters related to alkalinity, nitrite (NO_2-N), and nitrate (NO_3-N). Alkalinity was assessed through a titration method with sulfuric acid, TDS was determined from the mass of solids remaining as residue from a filtered water sample after oven drying at a defined temperature of 105°C or 180°C; nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) were evaluated by the absorbance of light by the colored compound formed after nitrite reacts with specific reagent, named sulfanilic acid. The absorbance is measured at a specific wavelength (543-540 nm) using a spectrophotometer. Everything related to SW, with the notable exception of water temperature, was recorded for three-day periods.

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Table 1: Formulation and analyzed nutrient composition of the experimental diets containing graded dietary protein levels for juvenile *Mugil cephalus* cultured at 15 ppt salinity

Ingredients (g)	Diets (Experimental groups)[1]			
	CP_{25}	CP_{30}	CP_{35}	CP_{40}
Fish meal	9.30	9.50	11.00	12.00
Soybean meal	9.00	10.00	11.60	16.50
Mustard oil cake	9.00	10.00	9.50	10.00
Corn gluten meal	8.50	12.00	17.50	17.80
De-oiled rice bran	16.00	16.00	12.00	11.00
Wheat flour	8.00	8.00	8.00	8.00
Casein[2]	0.40	0.40	0.40	0.40
Gelatin[2]	2.50	2.50	2.50	2.50
Starch[2]	15.00	12.10	11.00	8.00
Dextrin[2]	8.00	8.00	6.50	6.50
Fish oil[3]	4.00	1.00	4.00	2.00
Vegetable oil[4]	3.80	3.00	1.50	1.80
Vit -min mix[5]	3.50	4.00	2.00	1.50
CMC[6]	3.00	3.50	2.50	2.00
Total (g/kg)	100	100	100	100
Proximate Composition (g/kg on fed-basis)				
Moisture	65.4	65.9	71.2	68.3
Crude protein	24.7	29.7	33.0	39.0
Ether extract	6.6	6.5	6.5	6.1
Crude fibre	5.1	5.3	5.2	5.3
Total ash	13.8	14.1	13.9	15.0
Nitrogen-free extract (NFE)	24.8	34.2	38.0	41.8
Gross energy (GE) (kcal/100g)	440.92	420.88	448.27	444.60
Digestible energy (DE) (Kcal/100g)	388.76	365.75	389.22	383.61
P:E[7] (g protein/MJ DE)	15.67	19.70	21.62	24.94

Notes: [1]CP_{25} (25 g/kg dietary crude protein); CP_{30} (30 g/kg dietary crude protein); CP_{35} (35 g/kg dietary crude protein); CP_{40} (40 g/kg dietary crude protein); [2]Ingredients procured from Himedia Pvt. Ltd., India; [3]Procured from Seacod Oil by Sanofi India Ltd., India; [4]Purchased from local retail shop [5]Composition of vitamin-mineral mixture (quantity/kg): Vitamin A, 550,000 IU; Vitamin D3, 110,000 IU; Vitamin B2, 2000 mg; Vitamin E, 750 mg; Vitamin K, 1000 mg; Vitamin B6, 1000 mg; Vitamin B12, 6 mcg; Calcium Pantothenate, 2500 mg; Nicotinamide, 10g; Choline Chloride,150 g; Mn, 27,000 mg; I, 1000 mg; Fe, 7500 mg; Zn, 5000 mg; Cu, 2000 mg; Co, 450 L-lysine, 10 g; DL-Methionine, 10 g; Selenium 50 ppm [6]Carboxymethyl cellulose, purchased Himedia Pvt. Ltd., India; [7]Protein to energy ratio.

Proximate formulation

The entire body of the mullet, along with the proximate composition of the experimental diets, was reviewed using the AOAC's standard operating protocols (AOAC, 2023). Dry matter (DM) was determined using a hot air oven, where samples were weighed before and after drying, and DM was calculated by subtracting the moisture loss from the initial weight. Crude protein (CP) content was measured via a Kjeltec Kalipus 8100 (P. R. India) (method 990.03), and ether extract (EE) was determined via Soxtec SAS-AS (SOCSplus, 08, India) (method 920.39), and the ash content was measured via a muffle furnace (SISCO, 08, India) (method 942.05). The crude dietary fiber of the experimental diets was assessed by acid and alkali digestion of

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the specimens in Fibretec 202 (Tulin Equipment, India).

Procedures for dietary evaluation

Employing subtraction tactics, the diet's nitrogen-free extract (NFE) was calculated in the following manner:

$$\text{NFE}\left(\frac{\text{g}}{\text{kg}}\right) = 1000 - \left\{\text{CP}\left(\frac{\text{g}}{\text{kg}}\right) + \text{EE}\left(\frac{\text{g}}{\text{kg}}\right) + \text{CF}\left(\frac{\text{g}}{\text{kg}}\right) + \text{CA}\left(\frac{\text{g}}{\text{kg}}\right)\right\}$$

Determination of the diet's gross energy (GE) was performed using a Bomb Calorimeter (Changsu Instrument, China). The gross energy content of the simulated diets was specified in kilocalories per hundred grams. The following algorithms were incorporated to compute the feeds' digestible energy (DE) and protein-to-energy ratio (P: E):

$$\text{DE}\left(\frac{\text{Kcal}}{100\ \text{g}}\right) = \left\{\text{CP}\left(\left(\frac{\text{g}}{100}\right)\text{g} \times 5{,}65\right) + \text{EE}\left(\left(\frac{\text{g}}{100}\right)\text{g} \times 9{,}65\right) + \text{NFE}\left(\frac{\text{g}}{100}\text{g} \times 4{,}10\right)\right\}$$

$$\text{P}:\text{E}\left(\frac{\text{mg protein}}{\text{Kcal DE}}\right) = \frac{\text{CP}\left(\frac{\text{g}}{100}\text{mg as fed} - \text{basis}\right)}{\text{DE}\left(\frac{\text{Kcal}}{100\ \text{mg}}\text{as fed} - \text{feed}\right)} \times 1000$$

Ultimately, a percentage wet-weight basis was adopted to characterize the experimental mullet's whole-body nutritional composition.

Body metrics, survival rate, protein ingestion, and growth attainment

Before the start of the growth trial, 50 fish were randomly selected to measure initial weight. After 60 days, all the fish (having fasted overnight) were removed from the tank with a dip net and gently anesthetized with 50 mg of clove oil L^{-1}; their wet weight (g) was then determined to the nearest 1.0 mg. Five fish from each replicate per treatment were directly stored at -20°C for analysis of whole-body composition.

The parameters for body metrics, survival rate, protein ingestion, and growth attainment were calculated employing the following formulas:

$$\text{Weight gain (\%)} = \frac{\text{Final body weight (g)} - \text{Initial body weight(g)}}{\text{Initial body weight(g)}} \times 100$$

$$\text{Specific growth rate (\%)} = \frac{\ln \text{offinal wet weight} - \ln \text{of initial wet weight}}{\text{Duration of fedding trial in days}} \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Feed intake(dry weight in g)}}{\text{Wet weight gain (g)}}$$

$$\text{Feed efficiency (\%)} = \frac{\text{Weight gain (g)}}{\text{Total feed intake (g)}} \times 100$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Wet weight gain (g)}}{\text{Protein intake (dry weight in g)}}$$

$$\text{Lipid efficiency ratio (\%)} = \frac{\text{Wet weight gain (g)}}{\text{Lipid intake (dry weight in g)}}$$

$$\text{Survival (\%)} = \frac{\text{Total number of mullet harvested at the end of feeding trial}}{\text{Total number of mullet stocked at the commencement of feeding trial}} \times 100$$

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Statistical analysis

Data are presented as mean ± standard deviation (SD) of three replicate tanks per treatment. Before analysis, data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. Differences among dietary treatments were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test for multiple comparisons when significant differences were detected. A second-order polynomial regression analysis was performed to estimate the optimum dietary crude protein requirement based on weight gain (WG) and feed conversion ratio (FCR). Statistical analyses were performed using GraphPad Prism version 10.5 (GraphPad Software Inc., San Diego, CA, USA). Differences were considered statistically significant at $p<0.05$.

Results

The attributes and substances of water

Physical-chemical parameters, including pH, temperature, salinity, and dissolved oxygen, were found to vary between 8.12±0.11, 27.21±0.24°C, 15.45±0.15 g/L, and 5.47±0.09 mg/L for the qualitative groups, respectively, but there were no significant differences among treatments (Fig. 1).

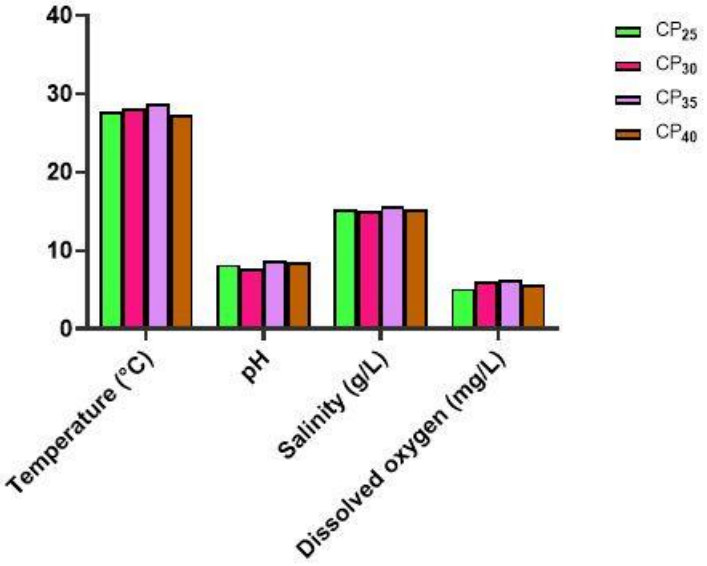


Figure 1: Water quality parameters of dietary crude protein requirements of mullet (*Mugil cephalus*).

No significant differences were found among treatments, and the highest values of NO_2-N and NO_3-N were demonstrated in the diet of CP_{35} (0.37±0.07 and 0.17±0.05 mg/L, respectively, whereas alkalinity values (142.1±1.47 mg/L) were superior in the CP_{30} (Table 2). Throughout the 60-day trial, no free CO_2 was detected in the water.

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Table 2: Water quality parameters of protein requirements of mullet (*Mugil cephalus*) for 60 days

Parameters	Diets			
	CP_{25}	CP_{30}	CP_{35}	CP_{40}
NO_2-N (mg/L)	0.35±0.02	0.33±0.10	0.37±0.07	0.34±0.03
NO_3-N (mg/L)	0.13±0.08	0.15±0.01	0.17±0.05	0.12±0.01
Alkalinity (mg/L)	140.23±1.05	142±1.47	137±1.21	140±1.58

Proximate composition of entire fish carcass and experimental diets

In the four experimental diets, corresponding to CP_{25}, CP_{30}, CP_{35}, and CP_{40}, protein levels were 24.7, 29.7, 33.0, and 39.0 g/kg. Apart from that, values for protein to energy ratio (P: E), crude fiber, total ash, gross energy (GE), digestible energy (DE), and ether extract (EE) oscillated between 15.67–24.94 g protein/MJ DE, 5.1–5.3 g/kg, 13.8–15.0 g/kg, 420.88–444.60 Kcal/100 g, 365.75–389.22 Kcal/100 g, and 6.1–6.6 separately (Table 1).

Whole-body composition of juvenile *Mugil cephalus* was significantly influenced by dietary protein level (Table 3). Whole-body crude protein content increased significantly (P=0.001) with increasing dietary protein, reaching the highest value in fish fed the CP_{35} diet (22.1±0.04%), followed by CP_{40}, CP_{30}, and CP_{25}. Similarly, whole-body lipid content increased significantly (P=0.001) with increasing dietary protein and was highest in the CP_{40} group (4.6±0.16%). In contrast, moisture and total ash contents were not significantly affected by dietary protein level (p>0.05), indicating that graded dietary protein primarily altered protein and lipid deposition without affecting body water or mineral content.

Table 3: Whole-body proximate composition (on g/kg dry weight basis) of mullet fingerlings cultured in saline water of 15 ppt and fed with hetero-nitrogenous experimental diets for 60 days.

Body composition parameters	Diets				*P*-value
	CP_{25}	CP_{30}	CP_{35}	CP_{40}	
Moisture	72.5±0.15	72.8±0.74	72.5±0.27	72.6±0.12	NS*
Crude protein	$18.9±0.12^{c}$	$20.8±0.45^{b}$	$22.1±0.04^{a}$	$21.3±0.15^{ab}$	0.001
Lipid or ether extract	$3.2±0.41^{c}$	$3.4±0.11^{c}$	$3.9±0.09^{b}$	$4.6±0.16^{a}$	0.001
Total ash	4.9±0.06	4.8±0.09	4.0±0.09	4.3±0.10	NS

Notes: Data are expressed as mean (n=3); Mean values in the same column with different superscripts differ significantly (p<0.05); *NS denotes not significant.

Growth efficiency and survivability

The growth performance of grey mullet fed diets with different crude protein levels is presented in Table 4. Fish fed the CP_{35} diet exhibited the highest final body weight (32.22±1.08 g), weight gain (325.06±1.32%), and specific growth rate (2.41±0.01%/day), together with the lowest feed conversion ratio (1.25±0.04), indicating superior growth and feed utilization (p<0.05). Growth performance improved progressively from CP_{25} to CP_{35} but declined slightly at CP_{40}, suggesting that dietary protein

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levels above 35 g/kg did not provide additional growth benefits. Initial body weight, feed intake, and survival were not significantly affected by dietary protein level (p>0.05), indicating comparable experimental conditions among treatments. The specific growth rate (SGR) of juvenile *Mugil cephalus* increased with dietary protein level up to the CP_{35} diet, which exhibited the highest median SGR among all treatments (Fig. 2). A slight decline in SGR was observed in fish fed the CP_{40} diet, indicating that increasing dietary protein beyond 35% did not provide additional growth benefits. These results suggest that the optimum dietary protein level for maximizing growth under 15 ppt salinity is approximately 35 g/kg (CP_{35}).

Table 4: Growth performance, feed utilization, and survival of juvenile *Mugil cephalus* fed experimental diets containing graded levels of dietary crude protein for 60 days at 15 ppt salinity.

Growth parameters	Diets				*P*-value
	CP_{25}	CP_{30}	CP_{35}	CP_{40}	
IBW (g)	7.56±0.06	7.51 0.07	7.58±0.02	7.55±0.05	NS*
FBW (g)	27.25±1.25^{c}	29.49±1.87^{b}	32.22±1.08^{a}	30.48±1.44^{ab}	0.078
Weight gain (%)	260.44±1.11^{c}	292.67±1.65^{b}	325.06±1.32^{a}	303.70±1.87^{ab}	0.023
SGR (%/day)	2.14±0.07^{c}	2.28±0.05^{b}	2.41±0.01^{a}	2.32±0.04^{b}	0.019
Feed intake (g/fish)	30.55±0.41	31.28±0.61	30.87±0.22	30.70±0.35	0.237
FCR	1.55±0.07^{a}	1.42±0.02^{b}	1.25±0.04^{c}	1.33±0.06^{b}	0.012
Survival (%)	93.33±2.44	96.56±1.54	97.23±2.71	95.55±2.10	0.341

Notes: Data are expressed as mean (n=3); Mean values in the same column with different superscripts differ significantly (p<0.05). CP_{25}-CP_{40}, 25-40 g/kg dietary crude protein; IBW, Initial body weight; FBW, Final body weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; *NS denotes not significant.

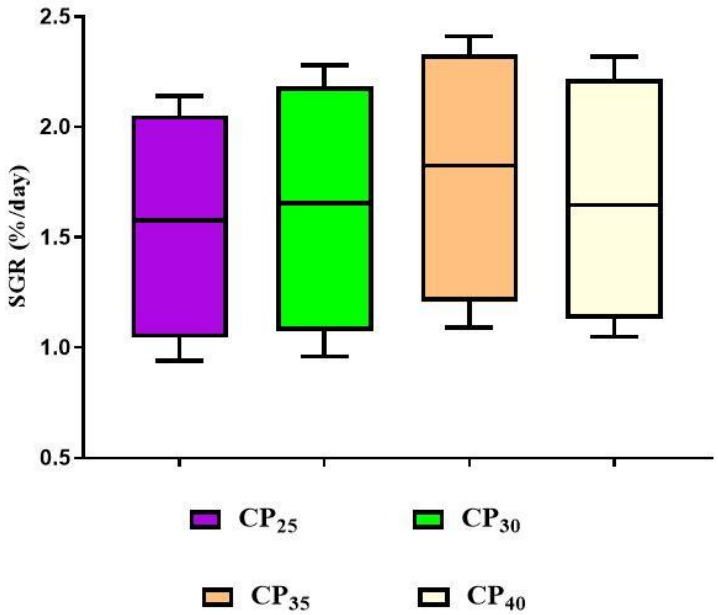


Figure 2: Comparison of production performance in terms of specific growth rate (SGR; %/day) of mullet, *Mugil cephalus,* for 60 days. Box-and-whisker plots with different superscripts indicating significant differences (p<0.05). A boxplot displays the median (horizontal line) and the upper and lower quartiles.

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Effects of crude protein on the feed utilization of grey mullet

Dietary protein level significantly influenced feed utilization indices of juvenile *Mugil cephalus* (Table 5). Fish fed the CP_{35} diet exhibited the highest feed efficiency (79.81±1.21%), protein efficiency ratio (PER; 2.29±0.04), and lipid efficiency ratio (LER; 7.11±0.58), which were significantly greater than those of the other dietary treatments (p<0.05). Feed utilization improved progressively with increasing dietary protein up to 350 g/kg but declined at 400 g/kg, indicating reduced nutrient utilization efficiency at excessive dietary protein levels. A significant increase was observed in PER values using 35 g kg^{-1} formulated diets, as compared to the other diets. The increase in crude protein linearly increased growth performance parameters. In addition, the WG (%) of grey mullet was positively correlated with the percentage of crude protein in the diet (R^2=0.9338) according to the following equation: WG (%) = $-0.0571x^2 + 4.1373x - 63.513$ (Fig. 3).

Table 5: Feed utilization efficiency of juvenile *Mugil cephalus* fed experimental diets containing graded levels of dietary crude protein for 60 days at 15 ppt salinity.

Items	Diets				*P*- value
	CP_{25}	CP_{30}	CP_{35}	CP_{40}	
Feed efficiency (%)	64.45±1.44^{c}	70.26±1.53^{c}	79.81±1.21^{a}	74.69±1.39^{b}	0.036
PER	1.97±0.05^{c}	2.10±0.08^{b}	2.29±0.04^{a}	2.13±0.02^{b}	0.022
LER	6.54±0.18^{bc}	6.96±0.05^{b}	7.11±0.58^{a}	6.22±0.08^{c}	0.010

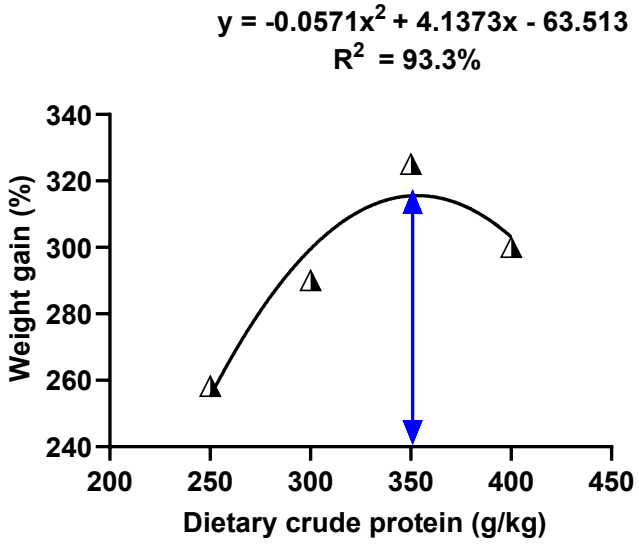


Figure 3: Optimization of dietary protein level of mullet based on weight gain (%).

There was a significant trend regarding the increasing crude protein for all feed and nutrient utilization, whereas the FCR was positively correlated (R^2=0.915) with CP_{35} levels (FCR=$0.0132\ x^2 + 2.255x - 0.805$) (Fig. 4). According to polynomial regression supervised by WG (%) and FCR, the maximum dietary crude protein dynamism of mullet juveniles raised in

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SW at 15 ppt salinity was 35 g kg^{-1} (Figs. 3 and 4). The effect size of one-way ANOVA, η^2=0.97, and the FCR $F_{(3,6)}$=57.61, p<0.001, 95% confidence interval for mean=3.72-5.09, declined significantly (p<0.05) due to rising dietary intake of protein up to 35.06 g/kg. When dietary protein consumption increased repeatedly, the FCR increased in the other approach.

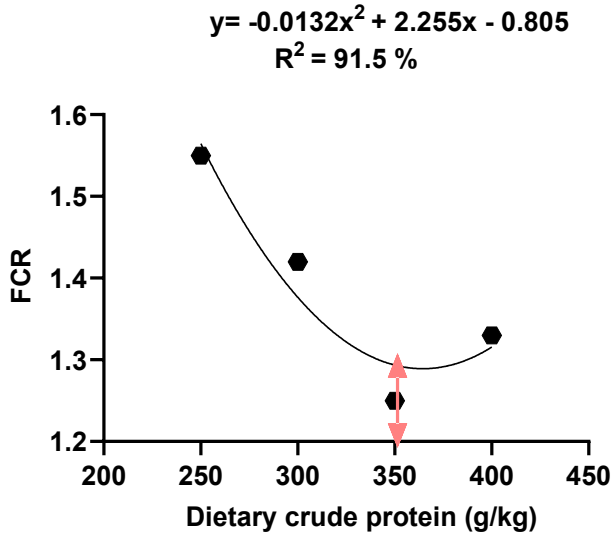


Figure 4: Optimization of the dietary protein level of mullet based on FCR.

Discussion

Following 60 days, the grey mullet fed 35 g protein/kg feed showed improvements in feed conversion ratio (FCR), lipid efficiency ratio (LER), weight growth (WG), final body weight (FBW), and SGR. According to these findings, CP_{35} g/kg promoted feed efficiency and growth reactivity. For the best potential growth and overall welfare of juvenile *M. cephalus*, most of the experimental groups' water parameters remained within the permitted limits as implied by Yones *et al.* (2019). Excess protein in the diet causes discomfort in aquatic life, leading to suboptimal growth, a greater tendency to disease, and ultimately, early death of the farmed animal (Kathyayani *et al.,* 2019). It also enhances the dispersion of nitrogen-containing compounds, such as ammonia, in the water (Mondal *et al.,* 2015). Ammonia lethality is closely associated with water pH; as the pH increases, it leads to a higher content of free ammonia (Kathyayani *et al.,* 2019). Other factors influencing ammonia toxicity include size, humidity, dissolved oxygen pattern, salinity intolerance, and respiration (Yones *et al.,* 2019). But both nitrite and nitrate are less hazardous to mullet than TA-N. Nevertheless, Mohamed and Abood (2019) observed that mullets succumb at nitrate concentrations exceeding 100 mg/L.

The whole-body proximate analysis of mullet revealed a significant increase (p<0.05) in body protein content, accompanied by a decrease in both

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moisture and ash levels as crude protein intake increased. Subbulakshmi and Sangeetha (2022) and Celik *et al.* (2012) independently reported the natural accumulation of epidermal fat in mullet larvae and juveniles. However, in contrast to our experiment, El-Dahhar (2000), Kumaran *et al.* (2012), and Alparslan *et al.* (2019) all concluded at the same time that it was difficult to significantly alter the juvenile *M. cephalus*'s total composition of crude fat and ash by giving it a specific amount of dietary protein. Mullet fed a crude protein of 35 g/kg showed higher digestibility due to a lower ash content than other diets, resulting in improved final growth. Additionally, Yones *et al.* (2019) illustrated that the crude fat implications of the previous investigation were slightly different from those of crude protein appraisals. Although the Bay of Bengal mullet fish had a consistent moisture content (69.7 g/kg), Ullah *et al.* (2022) noted that the crude protein (16.7 g/kg), ether extract (1.4 g/kg), and ash (1.7 g/kg) showed unevenness. The primary factors influencing the growth and dynamic development of fish and other wildlife are their diet and habitat (Paul *et al.,* 2022). A hypothetical dietary protein level surpasses the dietary lipid consideration by increasing the protein-to-energy ratio (P: E). This is because nearly all amino acids derived from food protein come into contact with muscle protein waste, which stimulates the emergence of fish with substantial nitrogenous waste in polluted areas (Zaman *et al.,* 2022). In this investigation, increasing dietary protein levels up to 35 g/kg, accompanied by a lower FCR, resulted in a substantial increase in the WG, SGR, and PER of mullet juveniles. However, above this level, it marked a significant decrease in growth and protein consumption, accompanied by an increased FCR. For mullet juveniles raised in SW at 15 ppt salinity, this finding clearly shows that feeding excess protein above the ideal level did not improve growth. This could be because excess protein with an unbalanced P: E ratio likely decreases the palatability and bioavailability of amino acids (Jana *et al.,* 2022).

Additionally, fish that consume excessive protein in their diet are likely to experience metabolic fatigue, leading to poor growth (Khan *et al.,* 2021). Yones *et al.* (2019) and El-Dahhar *et al.* (2014) stated that providing mullet with excessive protein without the correct protein-to-energy (P: E) ratio can hinder their growth. Consequently, high protein and P: E ratios in aquafeed promote successful aquaculture operations, reduce feed costs, and reduce nitrogenous outflows that degrade water quality, all of which help fish growth (Akhter *et al.,* 2024). According to the polynomial regression analysis of WG and FCR in our study, the preferred dietary protein requirement of the mullet juveniles identified in SW of 15 ppt was verified as 35 g/kg, respectively. Along with the average preliminary body weight of 30 g for mullet fingerlings, Yones *et al.* (2019) provided a standard crude protein diet for mullet (25%, 30%, 35%, and 40%). According to this

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experiment, a 35% crude protein diet resulted in greater final body weight, SGR, FCR, PER, LER, and feed efficiency values. Gisbert *et al.* (2016) exhibited the most impressive outcomes during 60 days. They modified the transition meals by substituting plant-based protein for fish meal and used 36% crude protein at 18 ppt salinity. In this study, mullets treated with crude protein improved growth performance and enhanced feed intake activity. The results showed marked improvements in the growth performance of different crude proteins in a dose-dependent manner. The results aligned with those of Yin *et al.* (2014) and Jiang *et al.* (2021), who reported enhanced growth performance in grouper and *Ictalurus punctatus*. The study also indicated enhanced feed utilization indices (FCR and PER), which may explain the improved growth performance in mullets resulting from dietary protein supplementation (35%). Although the results showed that dietary crude protein did not significantly affect feed intake, mullets fed a diet with 35% protein showed improved growth performance. These results indicate enhanced feed utilization by dietary protein in mullets, resulting in improved FCR and PER. Liu *et al.* (2021) reported a trial involving partial replacement of fish meal with soybean meal in *Liza haematocheila.* The results indicated WG% (483.22 %), SGR (3.12 %/day), and FCR (1.89), which is similar to our findings. Abdel-Mawla *et al.* (2023) fed the thin-lip *Liza ramada* a supplementary laminarian diet with superior SGR (2.62±0.03 %/day), FCR (1.20±0.04), and PER (2.78±0.08) values compared to this study. Another trial was carried out by Abo-Taleb *et al.* (2021), who fed the grey mullet with zooplankton biomass meal, and the feed efficiency percentage (71.06±1.57 %), FCR (1.41±0.09), PER (2.33±0.08), and LER (7.70±0.05) values were in accordance with the present study. Proper digestion of feed leads to regulated metabolic function, thereby enhancing physiological and immunological responses (Zaman *et al.,* 2026). As noted by Aboelyzed *et al.* (2024), fish meal was substituted for fermented rice bran in the grey mullet. The 100% replacement exhibited the lowest FCR values (2.22) and a significant SGR (1.18%/day) and PER (1.66). The current study demonstrated a significant improvement in feed utilization in mullets treated with 35% crude protein, as evidenced by enhanced PER and FCR. Implementing four diets with 32, 36, 40, and 44% crude protein and 14 ppt salinity, the protein requirements for fingerling grey mullet, *Mugil cephalus*, with an initial body weight of 5.5 g on average, were assessed (Al-Hamid, 2010). The diets were fed for 90 days. According to our research, diets containing 35 g/kg crude protein showed the best growth indicators, followed by those containing 40, 44, and 32 g/kg crude protein. In turn, this is the first assessment of the dietary protein requirements of juvenile *M. cephalus* in Bangladesh under 15 ppt of seawater.

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Conclusion

This study demonstrated that dietary crude protein level significantly influenced the growth performance and feed utilization of juvenile *Mugil cephalus* reared under 15 ppt salinity. Fish fed the diet containing 350 g/kg crude protein (CP_{35}) exhibited the highest final body weight, weight gain, specific growth rate, feed efficiency, protein efficiency ratio, and lipid efficiency ratio, together with the lowest feed conversion ratio, indicating that approximately 350 g/kg dietary crude protein is the optimum requirement for maximizing growth and nutrient utilization. In contrast, increasing dietary protein to 400 g/kg did not confer additional growth benefits, suggesting reduced efficiency of excess protein utilization. Scientifically, this study provides the first quantitative estimate of the dietary protein requirement of juvenile *M. cephalus* cultured at 15 ppt salinity under Bangladesh's coastal farming conditions, thereby addressing an important knowledge gap in the nutritional management of this euryhaline species. The findings offer valuable baseline information for species- and salinity-specific feed formulation. From a practical perspective, the recommended dietary protein level can support the development of cost-effective and nutritionally balanced feeds, improve growth performance while reduce feed costs. Furthermore, optimizing dietary protein is expected to decrease nitrogen excretion and improve nutrient utilization, thereby contributing to environmentally sustainable aquaculture and reducing the ecological footprint of coastal fish farming. Future research should investigate the interactions between dietary protein, lipid, and digestible energy, evaluate amino acid requirements, nutrient digestibility, and physiological responses under different salinity regimes, and validate the recommended protein level under commercial pond farming conditions to further optimize grey mullet production.

Ethical approval

The Committee on Human Research, Publications, and Ethics (BFRI/24/16) of the Bangladesh Fisheries Research Institute, Mymensingh, granted permission to execute the study. This paper does not contain any studies with human participants by any of the authors.

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Data availability

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, and will be available upon request from the corresponding author.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have

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appeared to influence the work reported in this paper.

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