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Original Article

Effects of dietary protein on growth and nutrient utilization in ornamental koi fish *Cyprinus rubrofuscus* (Lacepede, 1803)

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Abstract
Ornamental fish farming is an expanding sector and determining the ideal dietary protein for fish growth is crucial for the success of fish farming in developing countries. In this study, the impact of varying dietary protein (DP) levels (25, 30, 35, 40, 45 and 50%) on the growth and feed conversion ratio (FCR) of koi fish (*Cyprinus rubrofuscus*) were assessed. The fish fed twice daily for 90 days at a rate of 5% of their body weight and growth parameters and FCR measured every 15 days. Results revealed that the highest weight gain (170.98 g) observed with the 35% DP diet (D-3), while the lowest gain (103.98 g) occurred with the 45% DP diet (D-5). The 35% DP diet also produced the specific growth rate (0.59±0.10), protein efficiency ratio (PER) 2.27±0.27 and feed efficiency percentage (FE%) 60.57±4.40, all of which were significantly higher ($p < 0.05$) than those from the other diets. The best FCR value of 1.65±0.12 was also found in the 35% DP diet (D-3), which was significantly lower ($p < 0.05$) than the others. Nutritionally, moisture and ash content was significantly lower ($p < 0.05$) in D-3 as compared to other diets. Additionally, dietary protein levels had a significant effect ($p < 0.05$) on final weight, specific growth rate, protein efficiency ratio and protein feed of *C. rubrofuscus*. These findings offer important insights into the optimal dietary protein level for *C. rubrofuscus*, which could help improve the commercial success of ornamental fish farming.

Keywords: *Cyprinus rubrofuscus*; dietary protein; growth; koi fish; ornamental fish

1 | INTRODUCTION

The ornamental fish industry is a global market centered on the trade of both freshwater and marine species (Munguti *et al.* 2024). Often referred to as "living jewels," ornamental fishes are prize for their vibrant colors, various sizes and unique behaviors. Ornamental fishes are commonly kept in aquariums or garden ponds for decorative and recreational purposes (Hoseinifar *et al.* 2023; Munguti *et al.* 2024). Ornamental fish industry is a billion-dollar market, valued at $5.4 billion in 2021, with a projected annual growth rate of 8.5% through 2030 (FAO 2022; Hoseinifar *et al.* 2023; Munguti *et al.* 2024). Over 20 million ornamental fishes are estimated to be harvested from the wild each year and sold over two million aquarium enthusiasts worldwide (Chen *et al.* 2020). Approximately 5,000 freshwater species, 1,800 marine species traded globally as ornamental fishes (Evers *et al*. 2019; Novák *et al*. 2020; Munguti *et al*. 2024) and 90% of freshwater species breed in captivity (Hoseinifar *et al*. 2023). Around 80 countries participate in the export of ornamental fish, with Asian and developing nations dominating the industry (World Bank 2022).

Despite the significant economic value of ornamental fish, information about their nutritional needs, especially for those kept in aquariums, remains limited. Koi fish (*Cyprinus rubrofuscus*) is one of the most important freshwater ornamental fish species (Galib and Mohsin 2010; Galib *et al.* 2013). It valued for its vibrant colors, rapid growth rate and high market demand (Kurnia *et al*. 2024). *Cyprinus rubrofuscus* offer several advantages for aquaculture, including affordable koi fish seeds, large individual sizes, ease of cultivation, attractive colors and body patterns and a high selling price when they reach maturity (Kurnia *et al*. 2024). Koi fishes are cultivated properly as a high-value fishery commodity, so it is essential to carefully monitor cultivation techniques to ensure successful farming practices (Kurnia *et al*. 2024). Therefore, farming of Koi fish requires a well-balanced diet with an optimal energy-to-protein ratio at an affordable cost. The fish industry faces challenges in feed formulation, particularly in reducing production costs while promoting fish growth (Ali *et al*. 2024; Yılmaz *et al*. 2024; Wu *et al*. 2025). Fish growth is influenced by factors such as food intake, type of food, feeding frequency and nutrient absorption efficiency (Yılmaz *et al*. 2024; He *et al*. 2024). The feed conversion ratio (FCR), which measures the efficiency of converting feed into body weight, can be affected by feed composition and varies across the fish life cycle, with different amounts of feed required at juvenile and adult stages (Debnath *et al*. 2022; Ali *et al*. 2024; He *et al*. 2024).

Body size is commonly used to assess fish growth, as it serves as a direct indicator of growth. Body weight can be measured directly, quick and require minimal equipment. Additionally, body size (weight) is closely associated with other biological traits, including feeding capacity and survival rate (Hassan *et al*. 2021; Ishtiaq *et al*. 2024).

Protein plays a critical role in providing energy for growth (Debnath *et al*. 2022; Ali *et al*. 2024; Wu *et al*. 2025) and supply essential and non-essential amino acids, making it a key macronutrient for fish (Kim *et al*. 2012; Kim *et al*. 2016). Fish feed typically contains high-cost ingredients like protein (NRC 1993; Mohseni *et al*. 2013). To optimize aquaculture, it is essential to assess the optimal protein level that achieves high growth while minimizing feed costs (NRC 2011; Kim *et al*. 2016; Debnath *et al*. 2022; Ali *et al*. 2024). The dietary protein requirements for fish typically range from 30% to 55%, depending on species and protein sources (NRC, 1993; Kim *et al*. 2016; Debnath *et al*. 2022).

To the best of our knowledge, no study has yet addressed the growth, FCR, and proximate composition of Koi fish in Pakistan. Therefore, the primary goal of this study was to analyse the growth, FCR and proximate composition of Koi fish (*C. rubrofuscus*) when fed different levels of dietary protein.

2 | METHODOLOGY

2.1 Experimental design

Specimens of *C. rubrofuscus* fish, with a body weight (BW) ranging from 12 to 18 g and total length (TL) between 2.60 and 3.10 cm were collected from the Furqan Fish Aquarium Center in Sahiwal, Punjab, Pakistan. The fish were acclimated in glass aquaria for 90 days before the feeding trial. The trial conducted in six glass aquaria, each aquarium with 30 L capacity at Zoology Laboratory of Hizb -Ur- Rehman Islamic Science College, Kamalia Punjab, Pakistan. One hundred and twenty (120) fish stocked with 20 fish per aquarium and 2 replicates used.

Six different diets with varying levels of dietary protein (DP) were formulated: Diet-1 (D-1) with 25% DP, Diet-2 (D-2) with 30% DP, Diet-3 (D-3) with 35% DP, Diet-4 (D-4) with 40% DP, Diet-5 (D-5) with 45% DP, and Diet-6 (D-6) with 50% DP (Table 1). The proximate compositions of the feed evaluated followed the methods described by (NRC 1993). The fish fed for 90 days at a rate of 5% of their body weight, divided into two equal meals per day. Body size (Body weight) serves as a direct indicator of growth and can be measured directly. Additionally, body weight is linked with feeding capacity and survival rate of fish species (Ishtiaq *et al*. 2024). Dissolved oxygen (DO) and pH levels of the water of each aquarium monitored daily and maintained temperature between 23-26° C throughout the trial. Physicochemical parameters such as pH, dissolved oxygen (DO) was measured with the help of HANNA HI 9828 and water temperature was monitored and maintained with RS Electrical RS 599 50 W Aquarium Submersible Immersion High Glass with Auto On/Off Thermostat Heater. Fish body weight (BW) and total length (TL) measured every 15 days for each *C. rubrofuscus* specimen to calculate the growth parameters listed in Table 2. At the end of the feeding trial, the survival rate of the fish in each aquarium evaluated according to the methods of (Sivaramakrishnan *et al*. 2022; Ding *et al*. 2022; Fan *et al*. 2021).

2.2 Proximate composition analysis

The whole fish body used for proximate composition analysis. At the end of the feeding trial, the fish euthanized by immersing them in a 250 mg L^{-1} MS222 solution for 10 minutes. After euthanasia, the *C. rubrofuscus* specimens dried in an oven at 80°C until they reached a constant weight to measure moisture content. Ash content was determined by incinerating the specimens in a muffle furnace at 550°C for 12 hours. Fat content calculated by extracting the samples with a chloroform and methanol solution (1:2). Protein content derived by calculating the mass difference after accounting for other constituents, following the method outlined by (Ahmed *et al*. 2022).

TABLE 1 All diets proximate composition and percentage of feed ingredients.

Ingredients	D1 (25% DP)	D2 (30% DP)	D3 (35% DP)	D4 (40% DP)	D5 (45% DP)	D6 (50% DP)
Fishmeal	10	10	15	21	30	37
Rice polish	25	15	10	4	3	0
Soybean meal	10	15	25	31	40	44
Canola meal	5	5	5	5	5	2
Wheat bran	15	19	10	4	3	2
Sarson meal	5	2	5	5	3	5
Sunflower meal	5	5	5	5	3	2
Corn gluten meal 30%	10	9	5	5	3	0
Corn gluten meal 60%	10	15	15	15	5	3
Soybean oil	2	2	2	2	2	2
Vitamin premixes	1	1	1	1	1	1
Carboxymethyl cellulose (CMC)	1	1	1	1	1	1
Dicalcium phosphate	1	1	1	1	1	1
Proximate body constituent analysis						
Moisture content	7.82	8.89	5.46	9.04	9.24	9.4
Dry matter (DM)	92.18	91.11	94.54	90.96	90.76	90.6
Dietary protein (DP)	26.35	34.36	45.28	35.81	32.15	34.2
Crude fat (CF)	11.86	7.4	6.15	9.86	9.2	8.45
Ash	9.25	9.42	8.14	9.91	8.7	8.4
Fiber	8.19	8.24	5.35	8.8	9.7	9.45
Nitrogen free extract (NFE)	44.35	40.58	35.08	35.62	40.25	39.5

NFE = DM – (%DP + %CF + %Ash + %Fiber)

2.3 Data analysis

ANOVA followed by Duncan's Multiple Range Test used to assess significant differences ($p < 0.05$) between the mean values of each treatment. This analysis conducted using SPSS version 23 to compare the diets D-1 to D-6 (Ahmed *et al*. 2022).

3 | RESULTS

Koi fish (*Cyprinus rubrofuscus*) growth performance was evaluated based on diets with varying dietary protein (DP) levels: 25% in Diet-1 (D-1), 30% in Diet-2 (D-2), 35% in Diet-3 (D-3), 40% in Diet-4 (D-4), 45% in Diet-5 (D-5), and 50% in Diet-6 (D-6) (Table 2).

TABLE 2 *Cyprinus rubrofuscus* growth performance summary of diets D-1 to D-6.

Parameters	Initial weight	Final weight	Weight gain	Weight gain %	Food fed	FCR	SGR	PER	FE %	Protein feed	Survival rate (%)
D-1	41.11± 47.16^{c}	58.02± 67.39^{c}	16.91± 20.23^{b}	41.19± 2.79^{b}	41.28± 48.48^{a}	2.42± 0.23^{bc}	0.32± 0.09^{b}	1.28± 0.06^{b}	41.39± 4.00^{c}	12.90± 12.83^{b}	100
D-2	45.83± 56.03^{d}	65.21± 76.63^{d}	19.37± 20.59^{c}	44.73± 4.80^{c}	45.11± 48.48^{b}	2.33± 0.03^{b}	0.38± 0.08^{c}	1.58± 0.04^{c}	42.93± 0.52^{d}	12.07± 12.12^{a}	100
D-3	65.45± 88.40^{d}	96.41± 121.99^{d}	30.96± 33.59^{d}	56.31± 16.32^{d}	49.00± 55.48^{c}	1.57± 0.04^{a}	0.62± 0.11^{c}	2.40± 0.46^{d}	63.87± 1.44^{e}	12.72± 12.83^{b}	100
D-4	46.09± 54.41^{e}	65.73± 77.16^{e}	19.64± 22.75^{c}	45.29± 7.84^{c}	49.33± 54.77^{c}	2.52± 0.05^{c}	0.39± 0.10^{d}	1.57± 0.09^{c}	39.67± 0.73^{b}	12.39± 12.83^{a}	100
D-5	37.96± 39.78^{b}	52.33± 56.80^{b}	14.37± 17.02^{a}	37.57± 2.32^{a}	51.50± 58.30^{d}	3.61± 0.02^{d}	0.28± 0.07^{a}	1.14± 0.07^{a}	27.69± 0.19^{a}	12.39± 12.83^{a}	100
D-6	35.53± 40.27^{a}	49.83± 57.15^{a}	14.30± 16.89^{a}	39.99± 1.02^{b}	52.50± 58.30^{d}	3.74± 0.07^{d}	0.27± 0.07^{a}	1.11± 0.09^{a}	26.79± 0.50^{a}	12.39± 12.83^{a}	100
p-value	<0.001	<0.001	0.002	0.002	<0.001	0.002	<0.001	0.002	<0.001	<0.001	

The results indicated revealed that mean weight gain 30.96 ± 33.59 g was observed significantly highest ($p < 0.05$) fed with D-3 (35% DP) in *C. rubrofuscus*. The specific growth rate (SGR) was also significantly highest ($p < 0.05$) in D-3 (35% DP) with a mean value of 0.62 ± 0.11. The weight gain percentage was highest in D-3 (56.31 ±

16.32), while the lowest was in D-5 (37.57 ± 2.32). The total weight gain was highest in fish fed with D-3 (199.98 g) and lowest in those fed with D-6 (98.98 g). Fish fed with D-1 (25% DP), D-2 (30% DP), D-4 (40% DP), and D-5 (45% DP) recorded total weight gains of 116.12 g, 130.51 g, 131.98 g, and 101.18 g, respectively. Dietary protein significantly affected the final weight, specific growth rate (SGR), protein efficiency ratio (PER), feed efficiency percent (FE%), and protein feed ($p < 0.05$) (Table 2).

The best food conversion ratio (FCR) was found in D-3 (1.57 ± 0.04), which was significantly lower ($p < 0.05$) than in the other diets. The highest FCR value was recorded in D-6 (3.74 ± 0.07). FCR values for the other diets were as follows: D-1 (2.42 ± 0.23), D-2 (2.33 ± 0.03), D-4 (2.52 ± 0.05) and D-5 (3.61 ± 0.02) (Table 2).

In terms of protein efficiency ratio (PER), D-3 had the highest value (2.40 ± 0.46), which was significantly higher ($p < 0.05$) than all other diets, while the lowest PER was recorded in D-6 (1.11 ± 0.09). The PER values for the remaining diets were; D-1 (1.28 ± 0.06), D-2 (1.58 ± 0.04), D-4 (1.57 ± 0.09) and D-5 (1.14 ± 0.07) (Table 2).

For feed efficiency percent (FE%), D-3 recorded the highest value (63.87 ± 1.44), which was significantly ($p < 0.05$) greater than the other diets, while D-6 had the lowest FE% (26.79 ± 0.50). The FE% values for the other diets were, D-1 (41.39 ± 4.0), D-2 (42.93 ± 0.52), D-4 (39.67 ± 0.73) and D-5 (27.69 ± 0.19) (Table 2). Finally, the survival rate of *C. rubrofuscus* was 100% across all diets (D-1 to D-6).

ANOVA results showed significant differences among all body constituents; moisture content, dry matter (DM), dietary protein (DP), crude fat (CF), ash, fibre and nitrogen-free extract (NFE) across diets D-1 to D-6. Moisture and ash content were significantly ($p < 0.05$) lower in D-3 (35% DP) compared to D-1, D-2, D-4, D-5, and D-6. Dietary protein (DP) content was significantly ($p < 0.05$) higher in D-3 (35% DP) than in the other diets. Fat content was also significantly ($p < 0.05$) lower in D-3 (35% DP) compared to D-1, D-2, D-4, D-5 and D-6. A summary of the proximate body constituents for diets D-1 to D-6 provided in Table 3.

The water quality of each aquarium was monitored by measuring temperature and pH twice daily at 7:00 AM and 5:00 PM, while dissolved oxygen levels were recorded once a week. The average values from these measurements are summarized in Table 4.

TABLE 3 Proximate body constituent analysis of *Cyprinus rubrofuscus*.

Parameter	Moisture content	Dry matter	Dietary protein	Crude fat	Ash	Fiber	Nitrogen free extract
D-1	7.82±0.49^{b}	92.18±0.49^{c}	26.35±0.14^{a}	11.86±0.28^{f}	9.25±0.07^{c}	8.19±0.12^{b}	44.35±0.76^{c}
D-2	8.89±0.28^{c}	91.11±0.28^{b}	34.36±0.14^{c}	7.4±0.14^{b}	9.42±0.21^{c}	8.24±0.14^{b}	40.58±0.63^{b}
D-3	5.46±0.07^{a}	94.54±0.07^{d}	45.28±0.07^{e}	6.15±0.14^{a}	8.14±0.02^{a}	5.35±0.03^{a}	35.08±0.16^{a}
D-4	9.04±0.09^{cd}	90.96±.09^{ab}	35.81±±.28^{d}	9.86±0.14^{e}	9.91±0.14^{d}	8.8±0.14^{c}	35.62±0.70^{a}
D-5	9.24±0.07^{cd}	90.76±0.07^{ab}	32.15±0.07^{b}	9.2±0.07^{d}	8.7±0.14^{b}	9.7±0.35^{d}	40.25±0.63^{b}
D-6	9.4±0.07^{d}	90.6±0.07^{a}	34.2±0.07^{c}	8.45±0.14^{c}	8.4±0.07^{ab}	9.45±0.14^{d}	39.5±0.42^{b}
p-value	<0.001	<0.001	<0.001	0.002	<0.001	0.002	<0.001

TABLE 4 Water quality measurement.

Parameter	Results	Reference
Temperature (°C)	23–25	23–26
pH	7.1–7.3	7.1–7.5
DO (mg L^{-1})	5.5–6.3	5.5–6.5

Reference values are based on Aries *et al.* (2022)

In *C. rubrofuscus*, fortnightly results showed that the highest mean weight gain was recorded in the D-3 (35% DP) feed as 13.22 g, 17.79 g, 23.05 g, 29.96 g, 41.0 g and 60.725 g after 15, 30, 45, 60, 75 and 90 days respectively. The lowest fortnightly mean weight gain was observed in D-6 (50% DP), with gains of 4.985 g, 7.95 g, 10.29 g, 12.86 g, 20.86 g and 28.865 g after the same periods. Fortnightly mean weight gains in the other diets were as: D-1 (25% DP) 6.15 g, 9.11 g, 11.795 g, 16.465 g, 23.17 g and 34.76 g; D-2 (30% DP) 7.88 g, 10.995 g, 13.35 g, 18.965 g, 28.055 g and 37.0 g; D-4 (40% DP) 8.685 g, 11.25 g, 14.29 g, 17.56 g, 25.16 g and 40.865 g; D-5 (45% DP) 5.9 g, 8.25 g, 10.29 g, 12.86 g, 18.96 g and 29.965 g after 15, 30, 45, 60, 75, and 90 days respectively. Mean weight gain fortnightly comparison across D-1 to D-6 shown in Figure 1.

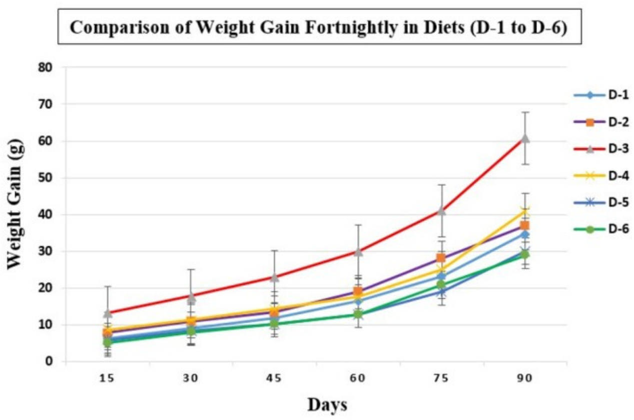

FIGURE 1 Mean weight gain fortnightly comparison across D-1 to D-6.

Fortnightly FCR results for *C. rubrofuscus* showed that the lowest mean FCR value was observed in D-3 (35% DP), with values recorded as 1.55, 1.56, 1.53, 1.53, 1.61 and 1.63 after 15, 30, 45, 60, 75 and 90 days respectively. In contrast, the highest mean FCR was recorded in D-6 (45% DP), with values of 3.91, 3.73, 3.82, 3.79, 3.64 and 3.53 at the same time points. The fortnightly mean FCR values for other treatments were as follows: D-1 (25% DP) showed 2.36, 2.22, 2.48, 2.54, 2.54 and 2.39; D-2 (30% DP) had 2.35, 2.20, 2.49, 2.36, 2.24 and 2.35; D-4 (40% DP) recorded 2.48, 2.55, 2.47, 2.61, 2.62 and 2.42; and D-5 (50% DP) had 3.65, 3.60, 3.62, 3.71, 3.63 and 3.47. Mean FCR fortnightly comparison across D-1 to D-6 shown in Figure 2.

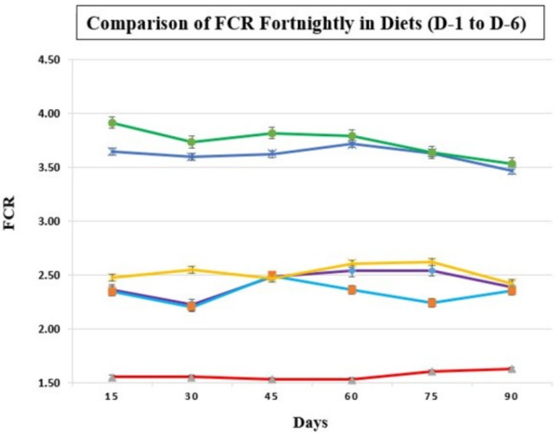

FIGURE 2 Mean FCR fortnightly comparison across D-1 to D-6.

Fortnightly SGR results for *C. rubrofuscus* showed that the highest mean SGR was observed in D-3 (35% DP), with values recorded as 0.88, 0.59, 0.51, 0.49, 0.54 and 0.67 after 15, 30, 45, 60, 75 and 90 days respectively. The lowest fortnightly mean SGR was recorded in D-6 (50% DP), with values of 0.33, 0.26, 0.22, 0.21, 0.27 and 0.32 at the same time points. The fortnightly mean SGR values for the other treatments were as follows: D-1 (25% DP) had 0.41, 0.30, 0.26, 0.27, 0.30 and 0.38; D-2 (30% DP) recorded 0.52, 0.36, 0.29, 0.31, 0.37 and 0.41; D-4 (40% DP) showed 0.57, 0.37, 0.31, 0.29, 0.33 and 0.45; and D-5 (45% DP) had 0.39, 0.27, 0.22, 0.21, 0.25 and 0.33. Mean SGR fortnightly comparison across D-1 to D-6 shown in Figure 3.

The results indicated that the highest mean weight gain of 30.96 g and the lowest mean FCR value of 1.57 were observed in D-3 (35% DP), while the lowest mean weight gain of 14.30 g and the highest mean FCR value of 3.74 were recorded in D-6 (50% DP). The comparison of mean weight gain and mean FCR values among D-1 to D-6 shown in Figure 4.

ANOVA analysis revealed significant differences in MC, DM, DP, CF, NFE and fibre among the diets (D-1 to D-6). Specifically, moisture content was significantly ($p <$ 0.05) lower in D-3 at 5.46 ± 0.07% compared to the other diets. Dry matter was significantly ($p < 0.05$) higher in D-3 at 94.54 ± 0.07%, while it was significantly ($p < 0.05$) lower in D-6 at 90.6 ± .07%. Dietary protein was significantly ($p < 0.05$) higher in D-3 at 45.28 ± 0.07%, whereas it was significantly ($p < 0.05$) lower in D-1 at 26.35 ± 0.14%. Ash content was significantly ($p < 0.05$) lower in D-3 at 8.14 ± 0.02%, while it was significantly ($p < 0.05$) higher in D-1 at 9.91 ± 0.14%. Proximate body constituent summary of D-1 to D-6 provided in Table 3.

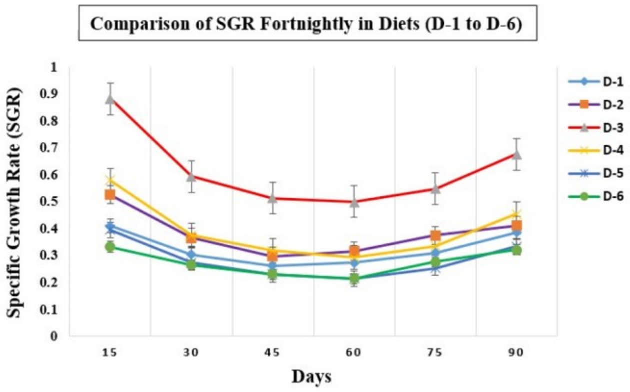

FIGURE 3 Mean SGR fortnightly comparison across D-1 to D-6.

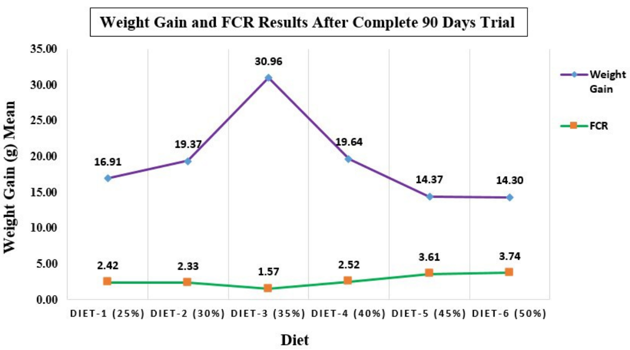

FIGURE 4 The comparison of mean weight gain and mean FCR values among D-1 to D-6.

4 | DISCUSSION

The optimal protein level for growth, feed utilization and body composition of *Cyprinus rubrofuscus* assessed in the current study. The highest specific growth rate (SGR), weight gain (WG) and the best feed conversion ratio (FCR) were observed in diets containing 35% protein, suggesting that this level is optimal for the growth of ornamental fish *C. rubrofuscus*. Lower growth rates and higher feed conversion ratios at protein levels exceeding this value can be attribute to the fish's inability to effectively utilize protein beyond the optimal threshold (Sivaramakrishnan *et al*. 2022). These results align with previous studies (e.g. Fan *et al*. 2021; Ding *et al*. 2022), which reported optimal protein levels of 35.1% for Crucian carp and 34.61% for common carp respectively.

In this study, the highest weight gain and weight gain percentage observed with a diet containing 35% protein in *C. rubrofuscus*. These findings are consistent with other reports (e.g. Ding *et al*. 2022; Sivaramakrishnan *et al*. 2022), who also found the highest weight gain in diets with 35.1% protein for ornamental fishes *C. auratus* and *Monodactylus argenteus* respectively. However, (Kim *et al*. 2016) reported the highest weight gain in ornamental fish (*O. fasciatus*) when fed diets containing 50 and 60% protein. The results of the current study suggest that a 35% dietary protein level is optimal for *C. rubrofuscus*.

The highest total weight gain 199.98 g recorded in *C. rubrofuscus* fed a diet with 35% dietary protein (DP) (Table 2). This result is in line with Ding *et al*. (2022), who reported the highest weight gain of 105.01 g in *C. auratus* fed a 35% protein diet. In contrast, Kim *et al*. (2016) found the highest total weight gain of 189 g in *O. fasciatus* fed a 50% DP diet. The study also found the lowest weight gain of 98.98 g in *C. rubrofuscus* fed a 50% protein diet, while Ding *et al*. (2022) observed a minimum weight gain of 98.76 g in *C. auratus* on a 30% protein diet. Similarly, Kim *et al*. (2016) reported the lowest total weight gain of 152 g in *O. fasciatus* fed a 35% DP diet.

The best feed conversion ratio (FCR) of 1.57 for *C. rubrofuscus* on a 35% DP diet found in present study, consistent with the findings of (Ding *et al*. 2022; Sivaramakrishnan *et al*. 2022), who reported the best FCR values of 1.29 in *C. auratus* and *Monodactylus argenteus*, respectively, on a 35% protein diet. The decreased growth (weight gain) and increased FCR beyond the 35% protein level suggest that fish may not efficiently utilize protein levels above this optimal amount (Sivaramakrishnan *et al*. 2022). These results support previous research on *C. auratus* and *Monodactylus argenteus*, where the optimal protein level was found to be 34.61% (Fan *et al*. 2021).

In this study, the protein efficiency ratio (PER) was found best for the 35% dietary protein diet. In comparison, Rahimnejad *et al*. (2015) reported PER for *E. fuscoguttatus* and *E. lanceolatus*. Kim *et al*. (2016) also identified 45%, 50%, and 60% dietary protein levels as optimal for the protein efficiency ratio in *O. fasciatus*.

The study also recorded a 100% survival rate across all diets containing 25, 30, 35, 40, 45 and 50% protein in *C. rubrofuscus*, aligning with findings from others (e.g. Kim *et al*. 2016; Ding *et al.* 2022; Belsare *et al*. 2024; Debroy *et al*. 2024) who also reported 100% survival in *O. fasciatus*, *C. auratus* and *H. jerdoni* when fed 30, 35 and 40% protein diets. The 100% survival rate observed in this study suggests that *C. rubrofuscus* has a high tolerance for confined systems and that the conditions were optimal for rearing (Farhat *et al*. 2011).

Regarding lipid content, the current study found that lipid levels in the fish increased with higher dietary protein, similar to the findings by Bai *et al*. (1999) for yellow puffer. This contrasts with Kim *et al*. (2012), who observed a decrease in whole-body lipid content with increased protein levels. While lipid content in this study was significantly influenced by dietary protein, these discrepancies could be due to differences in experimental conditions such as protein levels, fish species, feeding practices, stage of maturity, sex, condition factor, age, seasonal variations (Silva and Chamul, 2000) and body size (Karki *et al*. 2019).

The moisture content, dietary protein, crude fat and ash content were significantly influenced by dietary protein levels. In contrast, Ding *et al*. (2022) reported that these proximate composition parameters in *C. auratus* were not significantly affected by dietary protein levels, while Debroy *et al*. (2024) found significant effects of dietary protein on these parameters in *H. jerdoni*. The proximate composition of fish is influenced by factors such as species, size, dietary protein sources and environmental conditions (NRC 1993). However, the results of this study differ from those of other research on *Seriola dumerili* (Vidal *et al*. 2008), *Solea senegalensis* and *Siniperca scherzeri* (Valente *et al*. 2011).

While the high cost of fish feed with increased dietary protein presents a challenge for the commercial application of a 35% dietary protein diet, this diet is essential for improving fish growth and quality with a minimal feed conversion ratio. Further research is recommended to investigate the long-term effects of a 35% dietary protein diet on *C. rubrofuscus*, particularly its impact on reproduction and overall health.

5 | CONCLUSIONS

The current study shows that dietary protein levels have a significant impact on the growth, feed conversion ratio and proximate composition of ornamental koi fish *Cyprinus rubrofuscus*. The highest growth in ornamental fish observed when the diet contained 35% dietary protein (DP), compared to diets with 25, 30, 40, 45 or 50% DP. This suggests that a 35% protein inclusion in the feed is optimal for promoting growth and efficient feed utilization in *C. rubrofuscus*. Although there were some variations, the average protein percentages in the diets indicate that *C. rubrofuscus* is an excellent protein source for aqua-culturists. Furthermore, body size plays a crucial role in determining the fish's body composition. The results from this study will contribute to the development of nutritionally balanced diets for this ornamental species.

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ETHICAL STATEMENT

It has been confirmed that all animal care, handling and experiments were performed according to the guidelines of the Ethical Review Committee (ERC) (Animal Care and Use Committee), Pakistan. It is also declared that this study did not involve experiments on humans and no endangered or threatened species were used in this study.

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

Conceptualization: WA, MA & ZA, Methodology: WA, AA, NAK, MA & ZA, Sampling; WA, MA & ZA, Critical analysis and updating of the manuscript; WA, AA, NAK, MA & ZA, Writing – Original Draft Preparation: WA, Review & Editing: WA, AA, NAK, MA & ZA. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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