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Research Article

Effects of Varying Dietary Protein Levels on Growth Performance, Survival, Body Composition, Haemato-Biochemical Profile, and Metabolic Responses of *Hypselobarbus jerdoni* (Day, 1870) Juveniles

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A 60-day feeding trial has been carried out to access the optimal dietary crude protein (DCP) requirement of juvenile Jerdon's carp, *Hypselobarbus jerdoni*. Six isoenergetic (around 400 Kcal DE/100 g), isolipidic (60.80 g/kg), and heteronitrogenous diets were prepared with graded levels of protein, *viz*., 200, 250, 300, 350, 400, and 450 g/kg (TCP 20-TCP 45 with 50 g/kg incremental level). A completely randomised design (CRD) was used to distribute 270 Jerdon's carp juveniles (6.12–6.18 g) in six experimental groups in triplicates (15 fish/tank, 200 l water capacity). After 60 days, the fishes of the TCP30 group showed significantly higher weight gain, final body weight, and percentage weight gain (WG%). The fish of the TCP30 group exhibited a significantly higher feed efficiency ratio and specific growth rate. On the other hand, a significantly lower feed conversion ratio is recorded in the TCP30 and TCP35 groups. The protein efficiency ratio of fish was reduced significantly with the elevated DCP level. Whole-body moisture, lipid, and total ash contents of *H. jerdoni* were remain unaffected by DCP levels. However, DCP levels significantly influenced the whole-body protein of *H. jerdoni* juveniles, with significantly higher values noticed in the TCP30 and TCP35 groups. The TCP35, TCP40, and TCP45 groups exhibited significantly higher ($p < 0.05$) protease activity, and the amylase activity showed a decreasing trend in response to dietary protein levels. A significantly ($p < 0.05$) higher SOD and CAT activity were observed in the TCP20 and TCP25 groups. However, lower hepatic glutamate pyruvate transaminase and glutamic-oxaloacetic transaminase activity were observed in the TCP30 and TCP35 groups, respectively. Furthermore, based on broken-line linear and second-order polynomial regression with respect to WG%, the optimal dietary crude protein requirement of *H. jerdoni* cultured for 60 days was found to be 309.72 and 316.40 g/kg.

1. Introduction

Ornamental fishes are the most attractive and colourful pets, and keeping aquariums is the world's second preferred recreational activity after photography. Ornamental fisheries are generally considered as one of the most promising sectors within aquaculture that is rising day by day due to its great scope for enterprise development and income production. Moreover, the ornamental fish industry is popularly known as a multibillion-dollar industry wherein 125 countries are

engaged directly or indirectly in various trades [1]. According to the recent report, more than 2 billion live ornamental fish have been sold so far, with the global ornamental fish trade being valued around US$ 15–30 billion [2, 3]. In addition, the global ornamental fish trade involves roughly 2,500 species, of which more than 60% are freshwater species and the remaining species are marine. This industry is regarded as a sleeping giant in India. Being a tropical country, India is blessed with the vast biodiversity of freshwater ornamental fish and ample natural freshwater resources.

The Western Ghats are a line of hills that extend along India's west coast and are often regarded as one of the richest areas in the world in terms of biodiversity and endemism. The Western Ghats occupy a total area of 136,800 km^2 and span a length of 1490 km from north to south, with a width ranging from 48 km to 210 km [4]. According to a recent report, this biodiversity zone is home to 320 freshwater fishes from eleven orders, thirty-five families, and 122 genera, including numerous migratory species [5]. Of 320 species, 155 are recognized ornamental fishes, of which 117 are native to the Western Ghats [6]. The Western Ghats harbour several essential species, such as minor carps, barbs, minnows, loaches, rasboras, danios, and hill trouts, some of which are indigenous to this biodiversity hotspot region.

The medium-sized barbs of genus *Hypselobarbus* are popularly known as a "catchall" genus, which encompasses near about twenty-two species, and they are native throughout this biodiversity hotspot region of India, occurring primarily in the different stretches of the rivers and their connected reservoirs [7, 8]. This particular genus contains economically important ornamental, as well as food fishes that are sold in local markets [9]. These omnivorous, medium-sized fish showing potamodromous migration, i.e., migrate upstream for spawning during the rainy season [10]. Furthermore, the natural population of *Hypselobarbus* species is drastically declining as a result of the indiscriminate use of these species for aquarium and edible purposes in their native regions [11]. Owing to such combined concerns (territory loss and over exploitation), most of the species of *Hypselobarbus* are now addressed as "threatened" on IUCN Red List categorisation [12]. Among the twenty-two species, Jerdon's carp (*H. jerdoni*), locally known as Cha-meen, is a species of the Cyprinidae family endemic to India, mainly in the Western Ghat regions [7]. This species inhabits freshwater, is benthopelagic and potamodromous [13], and can grow to a maximum length of 46 cm (18 inch) in total length [14]. Moreover, as a consequence of the uncontrolled fishing and destruction of breeding ground due to anthropogenic activities, this species is now categorised as the "least concern" on the IUCN list [12]. Thus, there is utmost necessity of developing captive breeding protocols followed by subsequent closing of a life cycle and widespread culture of this species for purposes of conservation and sustainable trade.

Conservation efforts may be manifold, of which formulation of a nutritionally balanced and cost-effective feed is of utmost importance to spearhead captive maturation and breeding programmes. Fish growth results from a higher rate of body protein synthesis than breakdown, which is dependent on an optimal dietary protein supply and ambient conditions [15]. Among the several nutrients, dietary crude protein (DCP) is the most significant and costly ingredient in aquafeed, as well as a crucial component regulating fish growth [16]. Besides, dietary protein being of paramount importance should be supplied in adequate quantities through exogenous feed for fulfilling energy demands of the fish in various phases of its life. If the diet is low in protein compared to an optimum requirement level, it will reduce the growth rate of fishes [17–19]. Alternatively, if the diet is rich in dietary protein relative to the optimum level, then excess DCP will be used for the production of energy and simultaneously, fishes will excrete more ammonia into the water, which leads to poor growth in fishes [20]. Alternatively, if the fish diet contains more protein than necessary, not only does it pollute the environment by producing nitrogenous wastes but also raises the price of feed without providing any additional benefits for growth [21]. Therefore, dietary protein optimisation of a species at various life stages is critical towards making aquaculture lucrative and sustainable. Accordingly, various authors documented the optimum protein requirement of various barb species, such as 500 g protein/kg for tinfoil barb fry [22], 300–350 g protein/kg for lemon fin barb hybrid fingerlings [22], 350 g protein/kg for *Puntius vittatus* [23], 300 g protein/kg for *Puntius gonionotus* [24], 297 g protein/kg for *Labeo bata* fry [25], 281.48–282.53 g protein/kg for *Cirrhinus reba* fry [26], 250 g protein/kg for *Osteobrama belangeri* fingerlings [27], 290 g protein/kg goldfish fry [28], and 318.0–327.6 g protein/kg for *Hypselobarbus pulchellus* fingerlings [29]. In this regard, the present research explores the dietary protein requirement of Jerdon's carp (*H. jerdoni*) since it is a relatively new commercial ornamental fish, and many aspects of its culture including its food and feeding habit as well as nutritional requirements have not been examined yet. Likewise, no available information on the optimum DCP requirement at the early life stages (especially the juvenile nutrition with reference to protein) has yet been documented. In this context, the current research to determine the optimal dietary protein requirement of Jerdon's carp, *H. jerdoni* juveniles in relation to its growth performance, is justified for its conservation and efforts for its captive maturation and breeding.

2. Materials and Methods

2.1. Experimental Fish Procurement and Acclimatization. Six hundred and fifty (650) Jerdon's carp (*H. jerdoni*) fingerlings (weight 5.62–6.01 g; length 6.5–7.1 cm) were obtained from a small hatchery in Mangalore, Karnataka, India. Then, the fishes were carefully transported to the wet lab at the ICAR-Central Institute of Fisheries Education in Mumbai, India, in polythene bags comprising oxygenated water. On reaching the wet laboratory, the fishes were first treated with $KMnO_4$ solution (4 mg/L) for 2 min to remove the possible pathogens, and finally, mild salt (20 g/L) treatment was given for the duration of 2-3 min to overcome the transportation mediated stress. Following that, the treated fish were transferred to rectangular tanks (1.05 $m^2 \times 0.89$ m, 1000 L capacity) with vigorous aeration. The fishes were then acclimatized for

15 days in the same tank, and during this period, they were fed with 30% crude protein feed at satiation level twice daily. During the acclimatization period, 30% water was replaced with freshwater in every 2 days interval.

2.2. Formulation and Preparation of the Experimental Diets. Six heteronitrogenous (200–450 g CP/kg), isocaloric (around 400 kcal digestible energy/100 g), and isolipidic (60.80 g/kg) experimental diets containing graded levels of DCP, *viz*., such as 200, 250, 300, 350, 400, and 450 g CP/kg, were formulated and prepared (Table 1). Previously, dried ingredients were then crushed using a pulverizer, sieved, weighed, and securely stored in separate containers, according to the formulation. After adding the necessary amount of water, all the ingredients (excluding additives and oil) were mixed properly to make the dough. The dough was finally sealed in a heat-resistant polythene bag and steam-cooked inside a pressure cooker at least for 20 minutes, after which it was kept at room temperature for cooling. Then, the remaining ingredients, *viz*., vitamins-minerals mixture, betaine hydrochloride, oil, BHT, vitamin E, and Stay-C, were mixed properly with the cooled dough (cooked), and another dough was remade. The resulting dough was then put through a pelletizer to produce pellets (diameter of a pellet: 1.5 mm), which were then maintained at ambient temperature for 24 hours before drying in an oven (40°C) until the amount of moisture in the pellet went down below 100 g/kg (<10%) feed. At last, the dried pellets were packed into airtight polyethylene zipper bags and stored at 4°C till further use.

2.3. Setting Up of the Experimental Unit. Eighteen circular-shaped FRP (fibre glass-reinforced plastic) tanks with 300 L (1 m diameter × 0.8 m height, 200 L water volume) capacity were taken for the present study. The experimental tanks were disinfected with 4 ppm $KMnO_4$ solution followed by thorough washing with freshwater and finally dried well under sunlight. Following that, freshwater was introduced to the tanks, and continuous aeration was maintained. Then, using a completely randomised design (CRD), two hundred and seventy healthy and well-acclimatized fingerlings of Jerdon's carp (6.12–6.18 g) were dispersed randomly in six different treatments in triplicates using a stocking density (SD) of 15 fish/tank.

During the entire experimental period of 60 days, the animals were fed daily two times at 9: 00 am and 6: 00 pm with their respective diet to the apparent satiation level. Every morning, faeces from each tank were siphoned out, and the siphoned water was refilled with freshwater to maintain a uniform amount of water in all tanks. At every 14 days interval and after 60 days, the experimental animals from each tank were carefully weighed after subjecting to one day starvation for adjusting the satiation feeding level. Mortality of fish from every tank was counted daily during the entire experimental period to calculate the survival percentage. Throughout the 60-day experiment, a waterproof digital thermometer (Labart, India) and a pH probe (HANNA Instruments, Singapore) were used every day to measure the variety of water quality parameters, including temperature and pH, whereas dissolved oxygen, total hardness, total alkalinity, total ammoniacal nitrogen, nitrate-nitrogen, nitrite-nitrogen, and free carbon dioxide were measured at a 7-day interval following the standard protocols of APHA [30].

2.4. Sample Collection and Processing. Before starting of the feeding trial, initial body weight was measured. The survival percentage (%) was calculated by counting the living animals, and the final body weight of fish from each tank was assessed after a 24-hour fasting period following the conclusion of the feeding trial. Afterward, six fishes were randomly collected from each tank and anaesthetized with the help of clove oil solution (50 μl/L). After that, three fish were kept for carcass composition analysis, and the remaining three fish were thoroughly dissected to obtain liver, gill, muscle, and intestine samples. Following that, different organs were weighed separately and representative quantity were selected for homogenization with chilled sucrose solution (0.25 M) in a glass tube using a tissue homogenizer (REMI instrument) to prepare 5% tissue homogenates. Then, the 5% tissue homogenates were kept at 10,000 rpm for 15 min at 4°C for centrifugation (Thermo Fisher Scientific), and finally, the supernatants were collected in 2 ml vials and kept in a freezer (−20°C) for further analysis.

2.5. Proximate Analysis. Standard procedures were utilised to perform the proximate composition of six different experimental diets and whole carcass [31]. The amount of moisture in the samples was analysed with the help of oven drying at 102°C until they reached a constant weight. The remaining proximal components were calculated on a dry matter basis. The sample's nitrogen content was evaluated by using an automated micro-Kjeldahl analyser (PELICAN, India), and the CP level was obtained by multiplying the total nitrogen content by 6.25. Lipid content of the samples was calculated by following the method of solvent extraction. The Fibretec instruments (Tulin, India) were used to analyse the crude fibre of the diet samples. Furthermore, a muffle furnace was used to obtain the ash content of a sample (550°C for 6 hours, WIT; Australia).

2.6. Growth, Nutrient Utilisation, Survival Rate, and Body Indices. The growth and body indices were calculated using the following formulas:

Table 1: Formulation and proximate composition of the experimental diets (g/kg, dry matter basis).

Composition ingredients (g/kg)	Treatments[a]					
	TCP20	TCP25	TCP30	TCP35	TCP40	TCP45
GNOC[b]	205	205	205	205	205	205
Soybean meal	205	205	205	205	205	205
Casein	10	56	102	148	194.8	242
Gelatin	2.5	14	25.5	37	48.7	60.5
Starch	360	320	280	240	200	160
Dextrin	129.4	111.9	94.4	76.9	58.4	39.4
Fish oil	30	30	30	30	30	30
Veg oil	17.5	17.5	17.5	17.5	17.5	17.5
Vit. min. mix[c]	15	15	15	15	15	15
CMC[d]	20	20	20	20	20	20
BHT[e]	0.2	0.2	0.2	0.2	0.2	0.2
Betaine	5	5	5	5	5	5
Stay-C[f]	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin E	0.1	0.1	0.1	0.1	0.1	0.1
Total	1000	1000	1000	1000	1000	1000
Proximate composition (g/kg; on dry matter basis; mean of triplicates)						
Moisture	97.33	95.16	92.07	94.00	91.10	99.16
Crude protein	200.53	251.53	301.43	350.17	401.77	451.90
Ether extract	59.42	58.47	58.53	60.40	59.77	61.27
Total ash	43.10	40.90	40.17	45.27	45.20	48.60
Crude fibre	21.47	21.67	21.80	22.50	21.67	21.00
Nitrogen-free extract	675.48	627.43	578.07	521.67	471.60	417.23
DE (Kcal/100 g)[g]	403.88	404.21	404.48	403.09	403.14	402.79
P/E (mg CP/Kcal DE)[h]	49.65	62.23	74.52	86.87	99.66	112.19

[a]Treatment groups having graded levels of protein, i.e., TCP20 (200 g/kg dietary protein), TCP25 (250 g/kg dietary protein), TCP30 (300 g/kg dietary protein), TCP35 (350 g/kg dietary protein), TCP40 (400 g/kg dietary protein), and TCP45 (450 g/kg dietary protein). [b]GNOC: groundnut oil cake, procured from the local market, India. [c]Composition of vitamin-mineral mix (PRE-MIX PLUS) (quantity/kg): vitamin A, 5,500,000 IU; vitamin D3, 1,100,000 IU; vitamin B2, 2,000 mg; vitamin E, 750 mg; vitamin K, 1,000 mg; vitamin B6, 1,000 mg; vitamin B12, 6 mg; calcium pantothenate, 2,500 mg; nicotinamide, 10 g; choline chloride, 150 g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450 l-lysine, 10 g; DL-methionine, 10 g; selenium, 125 mg; vitamin C, 2,500 mg. [d]CMC: carboxymethyl cellulose, procured from HiMedia Ltd., India. [e]BHT: butylated hydroxytoluene, procured from HiMedia Ltd., India. [f]Stay-C 35, protected vitamin C. [g]DE, digestible energy (Kcal/100 g) = 4 × CP (g/100 g) + 9 × EE (g/100 g) + 4 × NFE (g/100 g) [45]. [h]P/E, protein-to-energy ratio (mg CP/kcal DE) = (CP% ×1000)/DE.

$$
\begin{aligned}
\text{weight gain (WG)} &= \text{final body weight (FBW)} - \text{initial body weight (IBW)}\\
\text{weight gain percentage (WG\%)} &= \frac{\text{(FBW-IBW)}}{\text{IBW}} \times 100\\
\text{specific growth rate (SGR)} &= [(\text{Ln FBW}) - (\text{Ln IBW})] \times 100\\
\text{feed conversion ratio (FCR)} &= \frac{\text{intake of feed in dry form (g)}}{\text{wet weight gain (g)}}\\
\text{feed efficiency ratio (FER)} &= \frac{\text{wet weight gain (g)}}{\text{intake of feed in dry form}}\\
\text{protein efficiency ratio (PER)} &= \frac{\text{wet weight gain (g)}}{\text{intake of protein in dry form (g)}}\\
\text{survival (\%)} &= \left(\frac{\text{animals harvested in live form}}{\text{animals stocked in live form}}\right) \times 100\\
\text{hepato - somatic index (HSI \%)} &= \left(\frac{\text{liver weight of the animal}}{\text{total weight of the animal}}\right) \times 100.
\end{aligned}
\tag{1}
$$

2.7. Enzyme Analysis

2.7.1. Tissue Protein Estimation. The amount of tissue protein in all the treatment samples was calculated by following the method of Bradford [32].

2.7.2. Digestive Enzyme Analysis. The techniques of Rick and Stegbauer [33] and Drapeau [34] were followed to measure the intestinal amylase and protease activity. Cherry and Crandall [35] techniques were followed to conduct the intestinal lipase enzyme activity.

2.7.3. Oxidative Stress-Related or Antioxidant Enzymes. The superoxide dismutase (SOD) assay was carried out using the Misra and Fridovich [36] method. The Takahara et al. [37] method was used to conduct the catalase (CAT) enzyme activity.

2.7.4. Protein Metabolic Enzymes. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in muscle and liver tissue homogenates were determined by using commercial kits from ERBA, India.

2.7.5. Carbohydrate Metabolic Enzymes. The Wroblewski and Ladue [38] method was used to evaluate the lactate dehydrogenase (LDH) activity. The malate dehydrogenase (MDH) activity was measured using the Ochoa [39] technique.

2.8. Haemato-Biochemical Parameters

2.8.1. Haematological Parameters. The Hendricks [40] method was applied to count red blood cells (RBCs) using a Neubauer hemocytometer. The Shaw [41] method was used to estimate white blood cells (WBCs) in fish blood samples. The van Kampen and Zijlstra [42] method was applied to estimate the haemoglobin (Hb) content in the blood samples. A haematocrit centrifuge was used to analyse the haematocrit value at 1000 g for 10 min [43].

2.8.2. Haemato-Biochemical Assay. Using commercial kits from ERBA, India, various haemato-biochemical parameters, including serum total protein and albumin, were determined. The following formulas were used to calculate globulin and the albumin-to-globulin ratio (A/G):

$$\text{globulin}\left(\frac{\text{g}}{\text{dl}}\right) = \text{serum total protein} - \text{serum albumin}$$
$$\frac{\text{A}}{\text{G}} = \frac{\text{serum albumin}}{\text{serum globulin}}. \quad (2)$$

2.9. Statistical Analysis. The experimental results were put through one-way ANOVA (analysis of variance) using the SPSS 22.0 statistical analysis tool to establish the means and average standard error of means (SEM). The overall treatment effects were evaluated, and then, the linear and quadratic effects of graded protein levels were measured using polynomial contrast analysis. Duncan's multiple range test with post hoc analysis was used to assess and determine the significant difference between means at the 5% probability level ($p < 0.05$). Finally, the data were presented as the means and average standard error of means (SEM). Furthermore, broken-line linear regression [44] and second-order polynomial regression analysis were conducted based on the WG% to optimise the DCP level [45].

3. Results

3.1. Physiochemical Parameters of Water. Different parameters relating to the water quality such as water temperature (°C), pH, total alkalinity (mg/L), total hardness (mg/L), dissolved oxygen (mg/L), total ammonia-N (mg/L), nitrite-N (mg/L), nitrate-N (mg/L), and free carbon dioxide (mg/L) of treatments were found in the ranges of 27.16–29.92°C, 6.99–7.44, 70.06–83.25 mg/L, 183.78–199.93 mg/L, 5.62–6.41 mg/L, 0.02–0.08 mg/L, 0.002–0.008 mg/L, and 0.03–0.09 mg/L, respectively, during the whole experimental period (Table 2).

3.2. Growth, Feed Utilisation, and Survival. The effect of varying DCP levels on growth, nutrient utilisation, and survival of *H. jerdoni* juveniles is presented in Table 3. Overall and quadratic trends of WG, WG%, and FBW were changed significantly ($p < 0.05$) among the treatments. These growth indices increased ($p < 0.05$) with the graded level of DCP up to 300 g/kg and showed a decreasing trend thereafter due to further increasing of DCP levels. With graded levels of DCP, the overall, linear, and quadratic trends of FCR and PER were significantly altered ($p < 0.05$). The FCR of the 300 g/kg protein-fed group was similar ($p > 0.05$) to that of the 350 g/kg protein-fed group and significantly lower than that of the rest of the groups. On the other hand, PER values reduced significantly ($p < 0.05$) with the increasing DCP levels. The overall and quadratic trends of SGR and FER were significantly ($p < 0.05$) influenced by DCP levels, with both parameters increasing significantly ($p < 0.05$) with increasing the DCP level up to 300 g/kg and significantly higher than the rest of the groups. As there was no mortality in any of the groups, all dietary groups had 100% survival.

3.3. Digestive Enzyme Activities. Though intestinal lipase activity did not differ substantially ($p > 0.05$) between treatments, graded amounts of DCP had a significant effect on the overall, linear, and quadratic trends of protease and amylase activities (Table 4). The amylase activity reduced considerably ($p < 0.05$) with the increased intake of DCP up to 400 g/kg and did not change further. On the other hand, the opposite trend was found in case of the protease activity, where it significantly enhanced ($p < 0.05$) with increasing the DCP level up to 400 g/kg and remained the same further in a high protein-fed group.

Table 2: Physicochemical parameters of water in different experimental units during 60 days of the feeding trial.

Parameters	[a]Treatments					
	TCP20	TCP25	TCP30	TCP35	TCP40	TCP45
Temperature (°C)	27.23–29.87	27.32–29.78	27.28–29.92	27.26–29.89	27.16–29.77	27.28–29.91
[b]DO (mg/l)	5.76–6.13	5.62–6.09	5.69–6.32	5.99–6.41	5.85–6.24	5.99–6.06
pH	7.02–7.05	6.99–7.23	7.03–7.43	7.08–7.11	7.12–7.44	7.00–7.32
Total alkalinity (mg/l)	71.23–82.23	70.21–79.82	71.65–80.33	73.24–81.76	70.06–81.55	70.11–82.35
Hardness (mg/l)	184.32–198.92	185.22–199.92	184.65–192.92	186.88–199.93	183.78–199.23	184.44–198.67
[c]TA-N (mg/l)	0.03–0.06	0.03–0.04	0.02–0.07	0.03–0.08	0.02–0.08	0.04–0.07
[d]NO^{-2}-N (mg/l)	0.002–0.004	0.003–0.005	0.003–0.008	0.006–0.008	0.002–0.006	0.002–0.005
[e]$N0^{-3}$-N (mg/l)	0.03–0.05	0.05–0.06	0.05–0.08	0.04–0.08	0.03–0.04	0.03–0.09
[f]Free CO_2 (mg/l)	ND	ND	ND	ND	ND	ND

[a]Treatment groups having graded levels of protein, i.e., TCP20, 200 g/kg protein; TCP25, 250 g/kg protein; TCP30, 300 g/kg protein; TCP35, 350 g/kg protein; TCP40, 400 g/kg protein; TCP45, 450 g/kg protein. [b]DO, dissolved oxygen. [c]TA-N, total ammonia nitrogen. [d]NO^{-2}-N, nitrite nitrogen. [e]$N0^{-3}$-N, nitrate nitrogen. [f]Free CO_2, free carbon dioxide. ND, not detected.

Table 3: Growth, nutrient utilisation, and percentage survival of *H. jerdoni* juveniles fed diets containing varying levels of dietary protein for 60 days.

Parameters	[a]Treatments						SEM	*p* values		
	TCP20	TCP25	TCP30	TCP35	TCP40	TCP45		Overall	Linear	Quadratic
[b]IBW	6.18	6.15	6.15	6.14	6.12	6.13	0.01	0.784	0.160	0.345
[c]FBW	11.50^{b}	12.07^{c}	13.22^{e}	12.62^{d}	11.87^{c}	11.02^{a}	0.18	0.000	0.342	0.000
[d]WG	79.87^{b}	88.85^{c}	106.09^{e}	97.20^{d}	86.26^{c}	73.37^{a}	2.68	0.000	0.387	0.000
[e]PWG	86.22^{b}	96.38^{c}	114.99^{e}	105.52^{d}	94.07^{c}	79.76^{a}	2.91	0.000	0.431	0.000
[f]FCR	2.69^{c}	2.39^{b}	2.21^{a}	2.30^{ab}	2.61^{c}	3.15^{d}	0.08	0.000	0.048	0.000
[g]SGR	1.03^{a}	1.13^{b}	1.28^{d}	1.20^{c}	1.10^{b}	0.98^{a}	0.02	0.000	0.430	0.000
[h]PER	3.99^{e}	3.55^{d}	3.54^{d}	2.78^{c}	2.16^{b}	1.63^{a}	0.20	0.000	0.000	0.000
[i]FER	0.37^{b}	0.42^{c}	0.45^{d}	0.43^{c}	0.38^{b}	0.32^{a}	0.01	0.000	0.091	0.000
Survival (%)	100	100	100	100	100	100	—	—	—	—

Mean values ($n = 3$) in the same column with different superscripts differ significantly ($p < 0.05$). [a]Treatment groups having graded levels of protein, i.e., TCP20, 200 g/kg protein; TCP25, 250 g/kg protein; TCP30, 300 g/kg protein; TCP35, 350 g/kg protein; TCP40, 400 g/kg protein; TCP45, 450 g/kg protein. [b]IBW, initial body weight. [c]FBW, final body weight. [d]WG, weight gain. [e]PWG, percentage weight gain. [f]FCR, feed conversion ratio. [g]SGR, specific growth rate. [h]PER, protein efficiency ratio. [i]FER, feed efficiency ratio.

Table 4: Digestive enzyme activities of *H. jerdoni* juveniles fed diets containing varying levels of dietary protein for the experimental period of 60 days.

[a]Treatments	[b]Amylase	[b]Protease	[c]Lipase
TCP20	1.85^{d}	53.24^{a}	4.90
TCP25	1.52^{c}	61.00^{b}	5.24
TCP30	1.47^{bc}	62.89^{bc}	4.11
TCP35	1.32^{b}	66.49^{cd}	4.78
TCP40	1.08^{a}	66.91^{d}	4.62
TCP45	1.09^{a}	67.24^{d}	5.28
SEM	0.07	1.26	0.21
Overall (*p* value)	0.001	0.001	0.680
Linear (*p* value)	0.001	0.001	0.878
Quadratic (*p* value)	0.001	0.001	0.529

Mean values ($n = 3$) in the same column with different superscripts differ significantly ($p < 0.05$). [a]Treatment groups having graded levels of protein, i.e., TCP20, 200 g/kg protein; TCP25, 250 g/kg protein; TCP30, 300 g/kg protein; TCP35, 350 g/kg protein; TCP40, 400 g/kg protein; TCP45, 450 g/kg protein. [b]Amylase activity is expressed as the micromole of maltose released/min/mg protein. [c]Protease activity is expressed as millimole of tyrosine released/min/mg protein. [d]Lipase activity is expressed as units/min/mg protein.

3.4. Carcass Composition and Body Indices. Whole carcass proximate composition and the body index values are depicted in Table 5. DCP levels had no significant ($p > 0.05$) effect on the whole-body moisture, lipid, and total ash contents of *H. jerdoni*. However, graded levels of DCP had a significant ($p < 0.05$) effect on the overall and quadratic trends of carcass protein, as well as the overall, linear, and quadratic trends of HSI in *H. jerdoni* juveniles. The fish fed with 300 g/kg protein (TCP30) and 350 g/kg protein (TCP35) showed significantly higher ($p < 0.05$) carcass protein than the other dietary groups. The HSI value significantly decreased ($p < 0.05$) with increasing DCP up to 300 g/kg, and beyond this, it was not affected significantly ($p > 0.05$) by increased dietary protein.

Table 5: Whole-body proximate composition (on g/kg, wet weight basis) and body indices of *H. jerdoni* juveniles fed diets with graded levels of dietary protein for the experimental period of 60 days.

Parameters	[a]Treatments						SEM	*p* values		
	TCP20	TCP25	TCP30	TCP35	TCP40	TCP45		Overall	Linear	Quadratic
Moisture	740.80	741.60	741.77	745.73	744.76	744.26	1.82	0.975	0.426	0.712
[b]CP	153.17[a]	156.50[a]	167.70[b]	165.07[b]	155.80[a]	157.49[a]	1.49	0.005	0.597	0.013
[c]EE	55.50	54.08	52.97	55.61	53.90	52.45	0.75	0.514	0.252	0.520
[d]TA	29.85	30.14	29.55	28.02	29.02	30.46	0.33	0.309	0.790	0.256
[e]HSI	1.43[b]	1.27[b]	0.92[a]	0.81[a]	0.78[a]	0.80[a]	0.07	0.001	0.000	0.000

Mean values ($n = 3$) in the same column with different superscripts differ significantly ($p < 0.05$). [a]Treatment groups having graded levels of protein, i.e., TCP20, 200 g/kg protein; TCP25, 250 g/kg protein; TCP30, 300 g/kg protein; TCP35, 350 g/kg protein; TCP40, 400 g/kg protein; TCP45, 450 g/kg protein. [b]CP, crude protein. [c]EE, ether extract. [d]TA, total ash. [e]HSI, hepato-somatic index.

3.5. Haematological Parameters. The haematological traits of fish fed with graded levels of DCP are displayed in Table 6. The concentration of haemoglobin and the number of white blood cells were not affected significantly across dietary groups, but the overall and quadratic trends of the RBC count and overall, linear, and quadratic trends of the haematocrit value were significantly affected ($p < 0.05$) by graded levels of DCP. The RBC count and the haematocrit value significantly increased ($p < 0.05$) with increasing the DCP level up to 300 g/kg, and a further increase in DCP significantly reduced ($p < 0.05$) the RBC count and the haematocrit value in fish.

3.6. Haemato-Biochemical Parameters. Overall, DCP levels affected the linear and quadratic trends of serum biochemical indicators significantly ($p < 0.05$) (Table 7). Serum total protein and albumin in fish fed with 200 g/kg protein (TCP20) and 250 g/kg protein (TCP25) were significantly lower ($p < 0.05$) than in other dietary groups. However, the total globulin level increased significantly ($p < 0.05$) as dietary protein levels increased to 300 g/kg and remained unchanged in afterward high protein-fed groups. The fish received 200 g protein/kg showed a similar P/E value ($p > 0.05$) in TCP25 and TCP30 groups and significantly higher ($p < 0.05$) than the other groups.

3.7. Oxidative Stress or Antioxidant Enzymes. In all, feeding of graded levels of DCP had a significant ($p < 0.05$) effect on the linear, quadratic, and overall trends of SOD and CAT activity (Table 8). The fish of TCP20 and TCP25 groups showed significantly ($p < 0.05$) higher SOD and CAT activity than those of other treatments.

3.8. Carbohydrate Metabolic Enzymes. Liver and muscle LDH and muscle MDH activities were significantly ($p < 0.05$) affected by graded levels of DCP overall, linearly, and quadratically (Table 9). The fish of TCP20 and TCP25 groups showed significantly ($p < 0.05$) higher hepatic and muscle LDH and muscle MDH activities than those of other dietary groups. However, the graded level of DCP did not affect hepatic MDH activity significantly ($p > 0.05$).

3.9. Protein Metabolic Enzymes. DCP levels had no effect on muscular AST and ALT activities ($p > 0.05$), whereas DCP levels had an effect ($p < 0.05$) on the overall and quadratic trends of hepatic AST and ALT activities (Table 9). The fish of TCP30 and TCP35 groups showed significantly ($p < 0.05$) higher hepatic AST and ALT activities than those of other dietary groups.

3.10. Optimum Dietary Protein Level. The optimal DCP requirement of *H. jerdoni* juveniles was evaluated using broken-line linear and second-order polynomial regression analysis, and the optimum DCP level of *H. jerdoni* juveniles was found to be 309.72 and 316.40 g/kg, respectively (Figure 1).

4. Discussion

4.1. Proximate Analysis of the Experimental Diets. The measured amounts of CP in several experimental diets, such as TCP20, TCP25, TCP30, TCP35, TCP40, and TCP45, were found to be 200.53, 251.53, 301.43, 350.17, 401.77, and 451.90 g/kg with corresponding P : E values of 49.65, 62.23, 74.52, 86.87, 99.66, and 112.19 mg protein/kcal DE, respectively. The crude protein, ether extract, and digestible energy (DE) [46] values of the various diets demonstrated that the diets were heteronitrogenous, isolipidic, and isoenergetic, supporting the premise of the protein requirement study [20, 47, 48].

4.2. Water Quality Parameters. Water quality parameters have a significant impact in maintaining physiological equilibrium in animals, including teleosts [48]. Among the several physicochemical properties of water, temperature (optimal 26–30°C) is the most critical factor in sustaining metabolic activity in cold-blooded animals including teleosts [49, 50]. Throughout the experiment, the temperature was kept between 27.16 and 29.92°C. Widiyati et al. [50] reported that the optimal pH range for a fish culture is 6–9, while the pH value fluctuated from 6.99 to 7.44 during the whole study. In the present experiment, the dissolved oxygen (DO) level was kept within a range of 5.62–6.41 mg/L, which falls closer to the optimal DO (>5 mg/l) level for fish production

Table 6: Haematological indices of *H. jerdoni* juveniles fed diets containing varying levels of dietary protein for the experimental period of 60 days.

Parameters	[a]Treatments						SEM	*p* values		
	TCP20	TCP25	TCP30	TCP35	TCP40	TCP45		Overall	Linear	Quadratic
[b]Hb (g/dl)	3.23	3.33	3.40	3.56	3.53	3.55	0.06	0.471	0.032	0.092
[c]RBC ($\times 10^6/\mu l$)	0.75^a	0.79^{ab}	0.90^c	0.91^c	0.83^b	0.84^b	0.02	0.001	0.088	0.002
[d]WBC ($\times 10^3/\mu l$)	23.60	24.99	25.27	24.66	25.24	24.89	0.33	0.768	0.343	0.395
[e]HCT (%)	12.33^a	12.97^a	16.23^c	16.34^c	14.93^b	14.90^b	0.39	0.000	0.014	0.000

Mean values ($n = 3$) in the same column with different superscripts differ significantly ($p < 0.05$). [a]Treatment groups having graded levels of protein, i.e., TCP20, 200 g/kg protein; TCP25, 250 g/kg protein; TCP30, 300 g/kg protein; TCP35, 350 g/kg protein; TCP40, 400 g/kg protein; TCP45, 450 g/kg protein. [b]Hb, haemoglobin. [c]RBC, red blood cell. [d]WBC, white blood cell. [e]Hct, haematocrit.

Table 7: Haemato-biochemical parameters of *H. jerdoni* juveniles fed diets containing varying levels of dietary protein for the experimental period of 60 days.

Parameters	Treatments						SEM	*p* values		
	TCP20	TCP25	TCP30	TCP35	TCP40	TCP45		Overall	Linear	Quadratic
Serum total protein (g/dl)	4.37^a	4.51^a	5.06^b	5.07^b	5.09^b	4.99^b	0.08	0.000	0.000	0.000
Albumin (g/dl)	1.89^a	1.90^a	2.12^b	2.09^b	2.08^b	2.05^b	0.03	0.005	0.009	0.002
Globulin (g/dl)	2.47^a	2.60^b	2.94^c	2.99^c	3.01^c	2.94^c	0.05	0.000	0.000	0.000
[b]A/G	0.77^b	0.73^{ab}	0.72^{ab}	0.69^a	0.69^a	0.70^a	0.01	0.003	0.003	0.003

Mean values ($n = 3$) in the same column with different superscripts differ significantly ($p < 0.05$). [a]Treatment groups having graded levels of protein, i.e., TCP20, 200 g/kg protein; TCP25, 250 g/kg protein; TCP30, 300 g/kg protein; TCP35, 350 g/kg protein; TCP40, 400 g/kg protein; TCP45, 450 g/kg protein. [b]A/G, albumin-to-globulin ratio.

Table 8: Oxidative stress enzyme activity of *H. jerdoni* juveniles fed diets containing varying levels of dietary protein for the experimental period of 60 days.

Parameters	[a]Treatments						SEM	*p* values		
	TCP20	TCP25	TCP30	TCP35	TCP40	TCP45		Overall	Linear	Quadratic
[b]SOD gill	9.84^b	10.24^b	7.60^a	7.09^a	7.62^a	7.51^a	0.31	0.000	0.000	0.000
[b]SOD liver	19.09^b	18.07^b	10.77^a	11.11^a	11.19^a	11.77^a	0.86	0.000	0.000	0.000
[c]CAT gill	4.85^b	4.75^b	1.70^a	1.71^a	1.85^a	1.69^a	0.35	0.000	0.000	0.000
[c]CAT liver	11.43^b	10.64^b	6.29^a	6.46^a	6.54^a	6.40^a	0.56	0.000	0.000	0.000

Mean values ($n = 3$) in the same column with different superscripts differ significantly ($p < 0.05$). [a]Treatment groups having graded levels of protein, i.e., TCP20, 200 g/kg protein; TCP25, 250 g/kg protein; TCP30, 300 g/kg protein; TCP35, 350 g/kg protein; TCP40, 400 g/kg protein; TCP45, 450 g/kg protein. [b]SOD, superoxide dismutase activity is expressed as 50% inhibition of epinephrine auto-oxidation/mg protein/min. [c]CAT, catalase activity is expressed as nanomoles of H_2O_2 decomposed/min/mg protein.

Table 9: Carbohydrate and protein metabolic enzyme activity of *H. jerdoni* juveniles fed diets containing varying levels of dietary protein for the experimental period of 60 days.

Parameters		[a]Treatments						SEM	*p* values		
		TCP20	TCP25	TCP30	TCP35	TCP40	TCP45		Overall	Linear	Quadratic
[b]GOT	GOT muscle	25.19	24.66	20.12	20.89	24.69	21.03	1.22	0.761	0.442	0.618
	GOT liver	18.06^b	18.22^b	15.41^a	15.59^a	18.65^b	18.15^b	0.35	0.000	0.794	0.021
[c]GPT	GPT muscle	24.85	26.19	21.84	19.73	27.83	26.23	1.23	0.482	0.719	0.466
	GPT liver	5.16^b	5.33^b	4.52^a	4.61^a	5.37^b	5.09^b	0.09	0.000	0.922	0.042
[d]LDH	LDH muscle	9.46^b	9.48^b	6.79^a	6.48^a	6.53^a	6.30^a	0.35	0.000	0.000	0.000
	LDH liver	2.37^b	2.17^b	0.97^a	0.92^a	0.85^a	0.78^a	0.16	0.000	0.000	0.000
[e]MDH	MDH muscle	1.53^b	1.61^b	0.62^a	0.64^a	0.56^a	0.50^a	0.12	0.000	0.000	0.000
	MDH liver	0.68	0.81	0.75	0.58	0.78	0.95	0.06	0.710	0.407	0.491

Mean values ($n = 3$) in the same column with different superscripts differ significantly ($p < 0.05$). [a]Treatment groups having graded levels of protein, i.e., TCP20, 200 g/kg protein; TCP25, 250 g/kg protein; TCP30, 300 g/kg protein; TCP35, 350 g/kg protein; TCP40, 400 g/kg protein; TCP45, 450 g/kg protein. [b]GOT, glutamate oxaloacetate transaminase activity is expressed as nanomoles of oxaloacetate released/min/mg. [c]GPT, glutamate pyruvate transaminase activity is expressed as nanomoles of sodium pyruvate released/min/mg protein. [d]LDH, lactate dehydrogenase activity is expressed in unit/mg protein/min. [e]MDH, malate dehydrogenase activity is expressed in unit/mg protein/min.

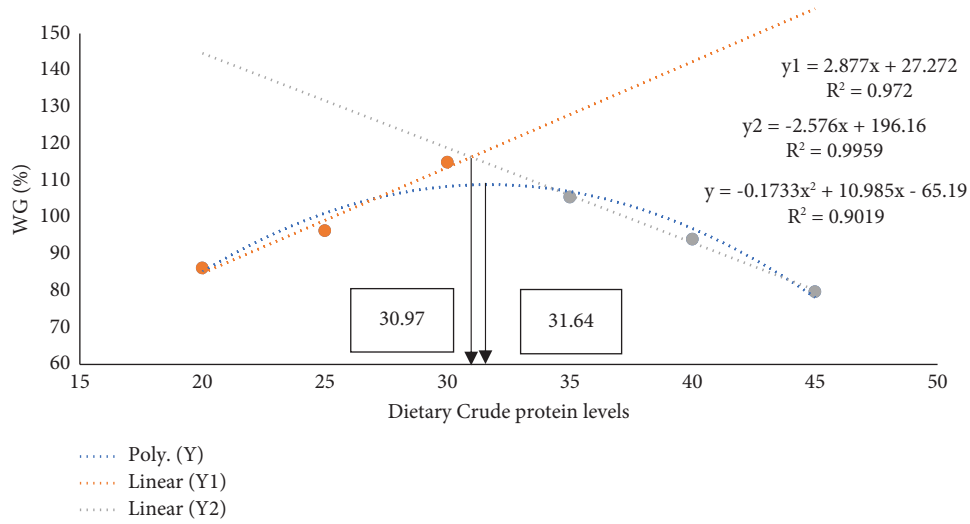

FIGURE 1: The broken-line linear and second-order polynomial regression analysis to optimise dietary crude protein requirement in relation to weight gain percentage (WG%) of *H. jerdoni* juveniles reared for 60 days and fed with graded levels of dietary crude protein.

[51, 52]. The recommended total alkalinity value for the fish culture, according to Santhosh and Singh [53], is 50–300 mg/L, and the total alkalinity (mg/l) value in the current study was kept within that range. The concentration of calcium (Ca^{+2}) and magnesium salts (Mg^{+2}), which are generally coupled with bicarbonates and carbonates (temporary hardness) and sulphates, chlorides, and other anions (permanent hardness) governs the total hardness of water [54].

In the present experiment, total hardness of different experimental units was observed within a range of 183.78–199.93 mg/L, which falls within that optimum range for the fish culture [55]. Total ammonium-nitrogen, nitrite-nitrogen, and nitrate-nitrogen concentrations were found to be within the acceptable range for aquaculture in the current experiment [56–59].

4.3. Growth and Nutrient Utilisation. In general, animal growth, including teleosts, is a phenotypic display of muscle hyperplasia that is influenced by various factors where environmental and nutritional factors are more crucial among them [48]. In a nutritional study, CP is the most vital dietary component in animals including fish as because its presence in the diet in adequate quantities not just supports growth but also delivers energy via amino acid breakdown (catabolism). Generally, many species growth is directly proportional to the DCP levels, but quantities of protein higher or lower than the optimum level cause growth retardation [60, 61] probably due to catabolism of more amino acids instead of body protein synthesis with reduced protein conversion efficiency in this situation at the cost of growth [62]. Furthermore, a lack of nonprotein energy in the diet lowered the fish growth performance despite having high protein in the diet probably due to preferential catabolism of dietary protein-derived amino acids as fish feed on energy satiation [63].

In the current experiment, different growth parameters such as WG, FBW, WG%, and SGR of *H. jerdoni* were observed to be raised with the raising levels of DCP up to 300 g CP/kg diet, but an increase in the DCP level in the diet reduces growth performance. This implies that excess dietary protein above the optimal amount with higher P : E values in the diet does not aid in growth rather assists in energy generation. Many other cyprinid species have shown growth retardation as a result of consuming protein at higher than optimal levels. Barlaya et al. [29] reported that of 20, 25, 20, 35, and 40% crude protein-fed groups, the peninsular barb *H. pulchellus* showed best growth in the 35% CP group at the end of 60 days. Similarly, other minor carp such as *C. reba* fingerlings showed best growth at 30% CP of 20, 25, 30, 35, and 40% CP-fed groups [26]. While rearing in flow through aquaria for 6 weeks, Lochmann and Phillips [28] reported that of 21.2, 25.3, 28.9, 31.1, and 34.5% crude protein-fed groups, the goldfish of the 28.9% CP-fed group exhibited the highest growth rate in terms of various growth indices.

The FCR and PER of the feed generally show how well the fish utilise the food item and the amount of protein it contains for growth. The FCR value reduced considerably ($p < 0.05$) with rising DCP levels up to 300 g CP/kg diet, but further addition of DCP in the diet increases the FCR of fish. Fish belongs to TCP30 (300 g CP/kg) and TCP35 (350 g CP/kg) groups with protein (P): energy (E) values of 74.52 and 86.87 mg protein/kcal DE, respectively, showing higher PER than in TCP40 and TCP45 groups. These data suggested that optimal dietary protein and P : E values may be a significant dietary element for effective protein utilisation, resulting in higher growth in fish. Similar kinds of results were also conveyed by Singha et al. [48], Santiago et al. [64], and Siddiqui et al. [65]. Moreover, Mohanta et al. [66] reported that silver barb showed lower FCR at the 30% CP level when fed with 20, 25, 30, 35, and 40% CP for a period of 90 days. In this study, PER is showing a declining trend with the escalation of protein in the feed which might be due to the reason that in lower protein-fed groups, probably almost all the dietary amino acids may be used in the formation of body proteins for achieving somatic growth. Alternatively, a significant proportion of dietary amino acids in the higher

protein-fed groups may be used for the production of energy rather than synthesis of body protein, resulting in lower PER. This finding was similar to the outcomes of other studies [29, 66].

4.4. Carcass Composition of Fish. The higher carcass CP content of *H. jerdoni* juveniles was observed in the TCP30 (300 g CP/kg) group than in the other protein-fed groups. Furthermore, when DCP levels exceeded 300 g/kg, there was a decreasing trend in body crude protein deposition. Many authors found similar patterns in carcass protein as a result of feeding varied levels of DCP [26, 29, 66]. The increasing trend of carcass protein of *H. jerdoni* juveniles up to an optimum level with an optimum P : E value demonstrated effective dietary protein utilisation for maximizing growth. However, the increasing level of DCP beyond an optimum level reduced growth probably due to excess amino acids produced from dietary protein used for the production of energy rather than synthesis of body protein that ultimately results in a decrease or no change in the carcass protein level. Our findings have been supported by Yadav et al. [26], who reported that the escalation of DCP up to 30% enhanced the CP deposition in carcass in case of *C. reba*. In this study, other parameters such as moisture, lipid, and ash remain unchanged ($p > 0.05$) among the different protein-fed groups. Similar inferences were also documented by many authors [26, 29, 67]. In this study, the graded level of DCP has no effect on the carcass lipid level which might be due to the effect of species differences or feeding isolipidic experimental diets. However, in contrast to the present study, Singh et al. [68] demonstrated the highest level of lipid deposition in fish of the optimum protein-fed group. The HSI value is directly proportional to the dietary energy sources and level as reported by many researchers [69, 70]. In this study, the HSI value of fish was significantly higher in lower protein-fed groups (TCP20 and TCP25) than in higher protein-fed groups. This disparity is most likely due to the reduction in digestible carbohydrate levels when protein levels in diets increase. It has been observed that lipogenesis from carbohydrate is a recognized biological phenomenon, where the excess dietary carbohydrate can be reserved as lipid in the liver, ultimately leading to an increase in the liver size or HIS [71, 72]. A similar kind of result was also demonstrated by several authors [73, 74].

4.5. Digestive Enzyme Activities. Digestive enzymes provide information about the whole digestion process and how efficiently fish digest the food item for their growth [75, 76]. The intestinal protease activity increased with rising DCP levels, which might be attributed to the availability of greater amounts of protein as a substrate in the intestine. The current finding supported the findings of Bazaz and Keshavanath [77] in *Tor khudree,* Singha et al. [48] in GIFT, and Jayant et al. [78] in *Pangasianodon hypophthalmus,* respectively. In case of amylase activity, a completely opposite trend was found; i.e., a negative correlation was evident between amylase enzyme activity with the DCP levels which may be attributed to less availability of starch or vice versa. Many authors have been found the similar kind of result [29, 78]. Finally, the DCP levels had no effect on intestinal lipase activity ($p > 0.05$) which might be attributed to feeding of all groups with isolipidic diets. This finding was as per the observation of Singha et al. [48] in GIFT and Barlaya et al. [29] in *H. pulchellus.*

4.6. Haematological Parameters. Haematological parameters are considered as vital indicators of fish health [79], and it also guides the biologist in identifying the physiological stress in fish caused by an abrupt changes in diet as well as in the environment [76, 80]. In this study, the RBC count showed an increasing trend with DCP levels up to 350 g/kg, which may have occurred due to its early release from the storage pool in the spleen [81, 82]. Then, again, a low RBC count probably caused less oxygen supply to the cells of a lower protein-fed group, which leads to poor growth in fishes [83]. Similarly, the haematocrit (Hct) value increased with increasing the dietary CP level up to 350 g/kg, which indicates the well transport of oxygen in the body, leading to improved fish health and growth [73]. On the other hand, the unchanged WBC count among different protein-fed groups in this study verified the results of Baruah et al. [84] and Kumar et al. [85] in rohu.

4.7. Serum Biochemical Profile. Serum biochemical indices (albumin, globulin, and serum total protein) constitute a dietary element that provides an excellent insight into a fish's immunological capacity under various environmental conditions [86]. The most important protein, albumin, is typically synthesised in the hepatocytes and plays a vital role in the transport of several essential biological components, including hormones, vitamins, and enzymes, as well as maintain the osmotic homeostasis in case of fishes [79]. On the other hand, globulins (gamma-globulins) help in maintaining the healthy immune system in case of teleosts [87]. The total protein, globulin, and albumin were significantly higher in moderate (TCP30 and TCP35) and high protein-fed groups (TCP40 and TCP45) than in low protein-fed groups (TCP20 and TCP25), which can be attributed to increased absorption of amino acids from protein digestion [88]. Many factors influence serum indices, including digestion efficiency, fish biomass, dietary composition, and temperature [89, 90]. A high protein level in the diet increases the activity of serum indices as well as protein metabolic enzyme function which generally reflects higher protein catabolism. Excess amino acids from protein-rich diets cannot be stored effectively in fish, but they can potentially be deaminated and transformed into energy molecules. [91]. In this current study, the increase in serum indices with dietary protein might be attributed to an increase in digested protein [92]. A similar kind of result was reported by Lu et al. [93] in red swamp crayfish. Moreover, a lower A/G ratio was observed in moderate and high protein-fed groups, which may be due to higher DCP levels in the diet which helps in terms of increasing the lymphocyte proliferation and subsequent immunoglobulin production in the body of fish and gives better immunological barrier to the fishes [94].

4.8. Antioxidant Enzymes. Continuous cellular metabolic activity produces free radicals or ROS, which cause oxidative stress, the breakdown of numerous macromolecules in cells, and tissue damage in animals [95]. The SOD-CAT enzymatic antioxidant mechanism is considered as the first line of defence against ROS in animals where SOD first encourages the dismutation of the superoxide anions (O_2^-) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) [96]. Following that, another enzyme catalase is responsible for the conversion of toxic hydrogen peroxide (H_2O_2) into H_2O and O_2. Therefore, higher activity of these enzymes denotes that animals are in a stressful condition. Accordingly, increased SOD and CAT activity in lower protein-fed groups (TCP20 and TCP25) in this study might indicate high dietary carbohydrate-driven stress in fish. In support with the current conclusion, higher SOD and CAT activities were recorded in GIFT of lower protein-fed groups [48, 79].

4.9. Activities of Carbohydrate Metabolic Enzymes. The MDH and LDH activities are closely related to anaerobic metabolism of carbohydrate and generally occur during the conditions of energy crisis, when malate is transformed into oxaloacetate and pyruvate into lactate (or vice versa), respectively, for giving energy via gluconeogenesis [97, 98]. In this study, higher LDH activity in low protein-fed groups (TCP20 and TCP25) might be attributed to high dietary carbohydrate-mediated oxidative stress in animals, which eventually triggers the anaerobic metabolism of pyruvate for energy production via lactate. Similarly, higher MDH activity in TCP20 and TCP25 groups might indicate the high carbohydrate-mediated oxidative stress with high-energy demand, which could be fulfilled via gluconeogenesis [98]. A similar kind of result was reported by many researchers, such as Singha et al. [48] in GIFT, Tok et al. [99] in *Pangasianodon hypophthalmus*, and Shimeno et al. [100] in *C. carpio*.

4.10. Activities of Protein Metabolic Enzymes. AST and ALT are the two transaminase enzymes that play an important role in amino acid metabolism [101]. In this experiment, the high hepatic ALT and AST activities in low (TCP20 and TCP25) and high (TCP40 and TCP45) protein-fed groups might indicate the synthesis of new amino acids, which, in former groups, probably took part in synthesis and accretion of body protein, but maybe due to insufficient dietary supply of amino groups, the sufficient quantity of new amino acids could not be synthesised to support optimum growth of fish; on the other hand, in latter groups, newly synthesised amino acids probably could be oxidized followed by gluconeogenesis for energy supply at the cost of growth of fish. However, the moderate activities of these enzymes in the fish of TCP30 and TCP35 groups indicate the synthesis of new amino acids, which probably could take part in synthesis and accretion of body protein to accelerate growth of fish. This finding might be due to dietary supply of optimum protein and protein-derived amino acids with an optimum dietary P : E ratio in the presence of sufficient energy from nonprotein sources (lipid and carbohydrate). The present findings corroborated the higher activities of hepatic ALT and AST in GIFT fed with varying dietary CP [48, 79].

4.11. Optimum Dietary Protein Requirement. In this study, using the broken-line linear and second-order polynomial regression analysis based on WG%, the optimum DCP requirement of *H. jerdoni* juveniles could be 309.72 and 316.40 g/kg, respectively, with the average value of 313.06 g/kg. This is the first research to indicate the optimal DCP requirement for *H. jerdoni* juveniles. Previously, various authors documented the optimum protein requirement of various barb species, such as 500 g protein/kg for tinfoil barb fry [22], 300–350 g protein/kg for lemon fin barb hybrid fingerlings [22], 350 g protein/kg for *Puntius vittatus* [23], 300 g protein/kg for *Puntius gonionotus* [24], 297 g protein/kg for *Labeo bata* fry [25], 281.48–282.53 g protein/kg for *Cirrhinus reba* fry [26], 250 g protein/kg for *Osteobrama belangeri* fingerlings [27], 290 g protein/kg goldfish fry [28], and 318.0–327.6 g protein/kg for *Hypselobarbus pulchellus* fingerlings [29].

5. Conclusion

In aquaculture, understanding the nutritional requirements of any species is critical for the formulation and preparation of cost-effective and environmentally friendly feed to optimise output in a sustainable manner. Based on broken-line and second-order polynomial regression analysis in relation to WG%, the optimum dietary crude protein levels for *H. jerdoni* juveniles reared for 60 days were found to be 309.72 and 316.40 g/kg. Furthermore, DCP within the 300–350 g/kg range gives a better condition for growth, nutrient utilisation, and physiological wellbeing of the fish. However, a further increment in DCP beyond the optimum level causes growth retardation in case of Jerdon's carp fingerlings. These observations will provide baseline data for developing nutritionally balanced diets for the intensive and semi-intensive culture of this fish species.

Data Availability

The data that support the findings of this study are accessible upon reasonable request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Subam debroy explored the study, confirmed data, built software programmes, and wrote the first draft. Paramita Banerjee Sawant designed the study, oversaw it, and evaluated and edited the report. Parimal Sardar created the approach, assisted with visualisation and validation, and read and edited the manuscript. Gouranga Biswas and Tincy Varghese contributed to the visualisation and validation, as well as editing the text. Mukunda Goswami and Debajit Sarma curated and validated the data. Manas Kumar Maiti, Ramjanul Haque, and Udipta Roy carried out formal analysis, software development, and data curation.

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