



Effects of Varying Dietary Protein Levels on Growth, Digestive Efficiency and Body Composition in Climbing Perch (*Anabas testudineus*) Fry

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ABSTRACT

Background: The present study was investigated to evaluate the effect of varying levels of dietary crude protein on growth, digestive enzyme activity, digestibility and whole body composition of *Anabas testudineus*.

Methods: Four iso-energetic diets were prepared at varying levels of dietary crude protein such as 25% (CP 25), 30% (CP 30), 35% (CP 35) and 38% (CP 38). A total of 300 numbers of fry (average fish weight - 0.56 ± 0.05 g) were stocked in 12 tanks (25 fish/tank), randomly in triplicate conditions and fed with experimental diets at ad-libitum level for 60 days.

Result: The study observed better growth performance and feed utilization in 35% and 38% crude protein fed groups. The highest weight gain (8.49 ± 0.08 g) was observed in CP 38 dietary group, while the highest apparent digestibility coefficient of dry matter ($72.24 \pm 0.27\%$), crude protein ($82.44 \pm 0.52\%$) and crude lipid ($84.59 \pm 0.62\%$) were also observed in the CP 38 dietary group. Protease activity was significantly ($P < 0.05$) higher in the CP 38 dietary group, whereas amylase activity was significantly ($P < 0.05$) lower in the CP 38 dietary group. Whole body composition improved with increasing protein levels, with the lowest crude protein content ($13.61 \pm 0.04\%$) in the CP 25 dietary group. To conclude, 35% crude protein is recommended as optimal for climbing perch fry due to its cost effectiveness, efficient nutrient utilization and reduced environmental impact.

Key words: *Anabas koi*, Body composition, Dietary protein, Digestive enzymes.

INTRODUCTION

Anabas testudineus (Climbing perch) is a freshwater fish with capable of air respiration commonly found in irrigation canals, lakes, ponds, agricultural fields, wetlands and estuaries and tolerate unfavorable environmental conditions with less oxygen that is usually prevalent in derelict waters bodies (Amornsakun *et al.*, 2005; Kumar *et al.*, 2013). Based on the dietary preferences and food choices, *A. testudineus* is categorized as omnivorous, although it has tendency towards a carnivorous diet (Mustakim *et al.* 2020). It fetches very good market price, especially in Southeast Asian countries such as India, Indonesia, Thailand and local markets of Northeast Indian states, where it is highly valued for its appealing flavor and rich nutritional content (Takagi *et al.*, 2011). Climbing perch is the potential candidate species for aquaculture diversification due to its exceptional physiological adaptation to various types of water bodies, including freshwater, brackish water and inland saline water (Talukdar *et al.*, 2021). Despite its advantages, culture of climbing perch has not been widely popularized due to non-availability of appropriate feed formulations tailored to its dietary needs.

Protein is a major and most expensive component in fish feed (NRC, 1993). Understanding the dietary protein requirement of climbing perch becomes a pre-requisite for the formulation of nutritionally balanced, efficient and cost effective feed for thriving aquaculture. Supplementation of optimal protein level in formulated diets is essential for

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sustainable aquaculture, as it minimizes nitrogenous waste output, improves feed efficiency and reduces production costs, ultimately promoting environmentally friendly and economically viable farming practices. Both insufficient and excessive protein in the diet not only influences fish growth and quality, but also impact water quality and overall expenditure of aquaculture (Ahmed and Ahmad, 2020).

Previous studies have indicated that the dietary protein requirement of climbing perch is ranged from 30% to 40% (Charoentesprasit and Jiwyam, 1996; Hossain *et al.*, 2012) and protein requirement vary according to fish size, source of dietary protein and environmental conditions (Yang *et al.*, 2003).

Digestive enzyme activities including amylase, protease and lipase are critical for nutrient digestion and absorption and their activities can indicate how well fish utilize dietary nutrients (Perez-Jimenez *et al.*, 2009). Additionally, analyzing whole body composition of fish serves as a useful tool for assessing the nutritional status and overall health of fish. Several studies reported that varying levels of dietary protein affected the digestive enzyme activity (Debnath *et al.*, 2007; Belsare *et al.*, 2024) and whole body composition (Belsare *et al.*, 2018; Surjobala *et al.*, 2021). Therefore, the present study was carried out to evaluate the effect of varying dietary protein levels on the growth performance, feed efficiency, digestive performance and body composition of *Anabas testudineus* fry.

MATERIALS AND METHODS

Experimental diet

Four iso-energetic diets were formulated with four different crude protein levels viz., 25% (CP 25), 30% (CP 30), 35% (CP 35) and 38% (CP 38). The ingredients and chemical

composition of experimental feeds are shown in Table 1. As per formulation, all the dry feed ingredients were mixed, cooked and pelletized as a 2 mm pellet using laboratory scale pelletizer. Subsequently pellets were oven dried at 45°C and stored in sealed air tight container.

Proximate composition of feed

The chemical composition of the experimental feed and whole body composition of fish was determined employing standard methods (AOAC, 2012).

Experimental design and feeding trial

The feeding trial was conducted at the Wet laboratory, Department of Aquaculture at Dr. M.G.R Fisheries College and Research Institute, Ponneri, Tamil Nadu, India during 2024. Climbing perch fry were obtained from Cochin Aquatics Fish Farm at Perumbavoor, Kerala. Prior to initiation of experiment fish were acclimatized to experimental conditions and fed with commercial feed for two weeks. 300 fries (average fish weight- 0.56 ± 0.05 g) were randomly stocked in twelve numbers of 450 L rectangular FRP tank. The experiment followed completely randomized design (CRD) in triplicate conditions and stocked fish were fed ad libitum level until visual satiation was noticed for 60 days. Tanks were siphoned out daily to remove the faecal matter before feeding. The physico-chemical properties of water were recorded daily following

Table 1: Formulation and chemical composition of the experimental diets with varying levels of dietary crude protein.

Ingredients	Protein levels			
	CP 25	CP 30	CP 35	CP 38
Fish meal ^a	130	220	330	400
Fermented Duckweed meal	26	44	66	80
Soybean meal ^b	120	120	120	120
Corn gluten meal ^c	90	90	90	90
Wheat flour	355	247	115	31
Cassava flour	154	154	154	154
Wheat bran	65	65	65	65
Soy lecithin	10	10	10	10
Fish oil	10	10	10	10
Vitamin premix ^d	10	10	10	10
Di-calcium phosphate	10	10	10	10
Starch	10	10	10	10
Chromium oxide ^e	10	10	10	10
Proximate composition (g/1000 g)				
Crude protein	255.1	300.5	350.2	381.9
Crude lipid	51.6	57.0	63.7	67.9
Crude fiber	30.3	34.7	40.1	43.4
Ash	73.8	99.0	129.9	149.5
Carbohydrate	587.9	507.6	415.1	356.2
Gross energy (Kcal/100 g)	434.21	431.91	428.25	425.89

^a, ^bDIVA, Muttukadu, Chennai, India. ^cHakita Feeds Pvt. Ltd., Kodambakkam, Chennai, India. ^dIndian Genomix Pvt. Ltd., Hyderabad, India. Composition of Vitamin-Mineral premix (quantity/kg): Vitamin A-700000 I.U, Vitamin D-70000 I.U, Vitamin E-250 mg, Nicotinamide-1000 mg, Cobalt-150 mg, Cu-1200 mg, Iodine-325 mg, Iron-1500 mg, Mg-6000 mg, Mn-1500 mg, Potassium-100 mg, Selenium-10 mg, Na-5.9 mg, S-0.72%, Zn-9600 mg, Ca-25%, P-12.75% ^eLoba Chemie Pvt. Ltd., Maharashtra, India.

standard protocol (APHA, 2012) and maintained under optimal levels by providing aeration and 50% water exchange on every alternate day. The recorded water quality parameters during the experiment were as follows; water temperature- $28.32 \pm 0.20^\circ\text{C}$; pH- 8.17 ± 0.04 ; dissolved oxygen- 5.92 ± 0.10 mg/l; alkalinity- 170.45 ± 1.34 mg/l; total ammonia- 0.02 ± 0.01 mg/l; nitrite- 0.09 ± 0.01 mg/l and nitrate- 0.03 ± 0.01 mg/l.

Growth performance

During the experiment, fortnight growth sampling was performed to assess the growth performance of the climbing perch. In each sampling, 100% of the stocked fish were collected and the individual total length and weight were recorded. Fish were subjected to 24 hours fasting period prior to sampling to ensure accurate measurements. The growth performance and feed efficiency indices were calculated as follows:

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)} \quad \dots(1)$$

$$\text{Specific growth rate (\%/day)} =$$

$$\frac{\text{Ln final weight of the fish} - \text{Ln initial weight of the fish}}{\text{Rearing period (days)}} \times 100 \quad \dots(2)$$

$$\text{Feed conversion ratio} = \frac{\text{Amount of feed consumed (g)}}{\text{Weight gain (g)}} \quad \dots(3)$$

$$\text{Feed efficiency ratio} = \frac{\text{Weight gain (g)}}{\text{Feed intake (g)}} \quad \dots(4)$$

$$\text{Protein efficiency ratio} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}} \quad \dots(5)$$

$$\text{Survival rate (\%)} =$$

$$\frac{\text{Number of fish survived at the end of the trial}}{\text{Number of fish stocked at the start of the trial}} \times 100 \quad \dots(6)$$

$$\text{Feed intake (FI)} = \frac{\text{Total amount of feed given}}{\text{Final fish population} \times \text{Duration (days)}} \quad \dots(7)$$

$$\text{Protein utilization value (\%)} =$$

$$\frac{\text{Final protein content of fish (g)} - \text{Initial protein content of fish (g)}}{\text{Total protein consumed}} \times 100 \quad \dots(8)$$

Digestive enzyme analysis

The estimation of total protein concentration was conducted using the Lowry's method (Lowry *et al.* 1951). Protease activity was measured according to the methodology outlined by Drapeau (1976). Amylase and lipase activity were analyzed by the protocols described by Rick and Stegbauer (1974) and Cherry and Crandall (1932), respectively.

Digestibility

Faecal collection

Faecal collection was initiated, one week after the start of the experiment, allowing adequate time for the fish to purge any remnants of the previous diet. One hour post feeding, excess feed was siphoned and removed. Two hours after feeding, uncontaminated faecal matter was siphoned and rinsed with distilled water. The faecal matter was then dried on filter paper at 40°C . Daily faecal samples were pooled to obtain an adequate quantity for analysis.

Determination of chromium content

Chromium content in the experimental diet and faecal matter was assessed to determine the digestibility of crude protein and crude lipid according to the methodology outlined by Furukawa and Tsukahara (1966).

Apparent digestibility coefficient (ADC)

The ADC of the experimental feeds was evaluated using chromium oxide (Cr_2O_3) as an inert marker at 1% of the diet according to pond *et al.* (1995) indicator method.

$$\text{ADC}_{\text{DM}}(\%) = 100 - \frac{\% \text{ indicator in diet}}{\% \text{ indicator in faeces}} \times 100$$

$$\text{ADC}_{\text{DIET}}(\%) =$$

$$100 - \frac{\% \text{ indicator in diet}}{\% \text{ indicator in faeces}} \times \frac{\% \text{ nutrient in faeces}}{\% \text{ nutrient in diet}} \times 100$$

Statistical analysis

To compare the significant differences among the treatments, One way Analysis of Variance (One-way ANOVA) followed by Tukey's test at a 0.05 significance level was performed using SPSS software, version 16.0. All the values are expressed as mean $\pm$ standard error ($M \pm \text{SE}$).

RESULTS AND DISCUSSION

Growth performance

Growth performance and feed efficiency of climbing fed with varying levels of dietary crude protein are shown in Table 2. In this present study, varying levels of dietary crude protein had a significant effect on growth rate, specific growth rate (SGR), daily growth coefficient (DGC), feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER). Our findings indicated that, diets with crude protein levels 35% and 38% demonstrated better growth performance and nutrient utilization compared to other treatments. The highest weight gain (8.49 ± 0.08 g) was observed in fish fed with 38% (CP 38) crude protein fed diet. In terms of SGR ($4.68 \pm 0.12\% \text{ day}^{-1}$) and DGC ($2.10 \pm 0.04\% \text{ day}^{-1}$), the highest values were recorded in the 35% (CP 35) crude protein fed diet. The growth rate of climbing perch increased progressively with increasing dietary protein levels from 25% to 38% crude protein containing diet. Although, the maximum growth parameters

were obtained when fish were fed with 38% crude protein containing diet, there were no significant differences was observed in growth parameters between the 38% (CP 38) and 35% (CP 35) diet fed groups. Therefore, the 35 % crude protein diet is considered as optimal due to its cost effectiveness, efficient nutrient utilization and reduced environmental impact.

Charoentesprasit and Jiwyam (1996) reported that a diet with 30% protein resulted in maximum growth of *Anabas testudineus*, while Hossain *et al.* (2012) found that 40% dietary protein is the optimal level for maximum growth in the climbing perch. The present study also found 35-38% crude protein as optimal level. No significant difference ($P>0.05$) was observed in the survival of fish fed with different crude protein diets, which is similar to the finding of Arshad Hossain *et al.* (2010) in *Pampus argenteus*. Feed intake in climbing perch showed no significant difference ($P>0.05$) among the dietary groups fed with different dietary protein levels, indicating that variations in protein content did not affect the feeding behavior of the climbing perch. However, some studies

reported that fish regulate feed intake based on protein intake rather than energy (Li *et al.*, 2017).

In the present study, feed conversion ratio (FCR) and protein efficiency ratio (PER) were recorded as suboptimal when the fish were fed with diets containing lower protein levels (25%, 30%). However, improvement in FCR and PER was observed with elevated levels (35% and 38%) of dietary protein in their diet. In addition, better FCR (1.37 ± 0.06) and PER (0.09 ± 0.003) were observed in fish fed with CP 35 diet. Similar trends of FCR and PER values were also reported by Kim and Lee (2009) in *Takifugu rubripes* and Ahmed and Maqbool (2017) in *Cyprinus carpio var specularis*. The enhancement of PER with each elevated protein level suggests that the appropriate protein level resulted in the highest PER, indicating the optimal protein availability for supporting the fish growth and maintenance (Ahmed and Ahmad, 2020). In the present study, CP 35 group exhibited a higher protein utilization value (PUV) ($30.33\pm1.19\%$), suggesting that this level of crude protein is optimal for climbing perch.

Table 2: Growth performance and nutrient utilization of climbing perch fed with experimental diets containing varying levels of dietary crude protein for 60 days.

Parameters	CP 25	CP 30	CP 35	CP 38	P value
Initial body weight (IBW g)	0.59 ± 0.02	0.57 ± 0.02	0.54 ± 0.04	0.55 ± 0.04	0.776
Final body weight (FBW g)	6.02 ± 0.09^c	6.66 ± 0.09^b	8.90 ± 0.07^a	9.04 ± 0.10^a	<0.001
Weight gain (WG g)	5.43 ± 0.11^c	6.09 ± 0.08^b	8.36 ± 0.10^a	8.49 ± 0.08^a	<0.001
Specific growth rate (SGR% day ⁻¹)	3.88 ± 0.08^b	4.11 ± 0.06^b	4.68 ± 0.12^a	4.67 ± 0.12^a	0.001
Daily growth coefficient (DGC% day ⁻¹)	1.63 ± 0.03^c	1.75 ± 0.02^b	2.10 ± 0.04^a	2.10 ± 0.03^a	<0.001
Survival rate (SR%)	92.00 ± 2.31	90.67 ± 1.33	93.33 ± 3.53	90.67 ± 3.53	0.891
Feed intake (FI g/ fish/day)	0.20 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.779
Feed conversion ratio (FCR)	2.16 ± 0.12^a	1.97 ± 0.07^a	1.37 ± 0.06^b	1.41 ± 0.04^b	<0.001
Feed efficiency ratio (FER)	0.47 ± 0.02^b	0.51 ± 0.02^b	0.73 ± 0.02^a	0.71 ± 0.02^a	<0.001
Protein efficiency ratio (PER)	0.08 ± 0.002^{bc}	0.07 ± 0.001^c	0.09 ± 0.003^a	0.08 ± 0.001^b	0.006
Protein utilization value (PUV%)	25.09 ± 1.32^b	23.96 ± 0.80^b	30.33 ± 1.19^a	27.27 ± 0.72^{ab}	0.011

Values are presented as Mean $\pm$ SE. Means with different superscripts in a row differ significantly ($P<0.05$).

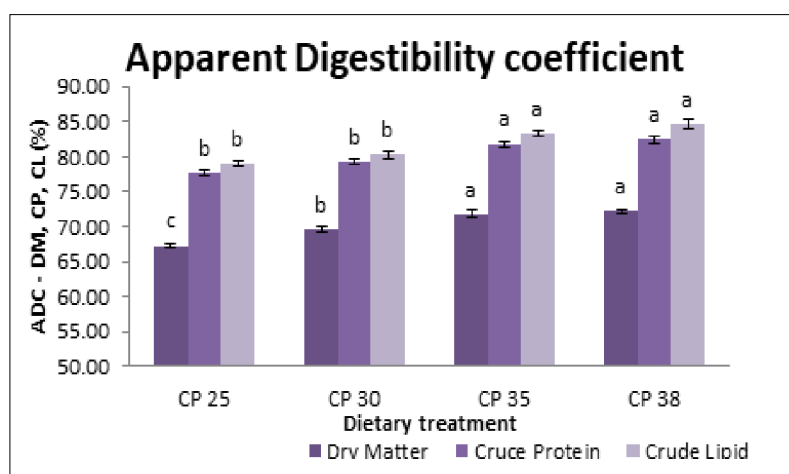


Fig 1: Apparent digestibility coefficient of climbing perch fed with varying levels of dietary crude protein

Apparent digestibility coefficient (ADC)

The apparent digestibility coefficient of climbing perch fed with varying levels of dietary crude protein is presented in Fig 1. Significant difference ($P<0.05$) was observed in the ADC of dry matter, crude protein and crude lipid of climbing perch fed with different diets. The highest values of ADC dry matter ($72.24\pm0.27\%$), crude protein ($82.44\pm0.52\%$) and crude lipid ($84.59\pm0.62\%$) were observed in CP 38 dietary group. However, ADC did not differ significantly among the fish fed with CP 35 and CP 38 dietary groups.

Apparent digestibility coefficients (ADCs) are becoming more widely used by aquaculture nutritionists and feed developers to assess the quality of diet ingredients and dietary formulations (Hardy, 2010). In the present study, the highest ADC of dry matter, crude protein and crude lipid observed in the groups fed with higher protein diets (35% and 38%), indicating that increased protein levels enhanced nutrient absorption and utilization. The lower ADC values in the lower protein fed groups (25% and 30%), indicating that inadequate dietary protein negatively impacts the fish's ability to digest and absorb nutrients effectively. These findings highlight the importance of formulating diets

with optimal protein levels to maximize growth performance and overall health in climbing perch. Similar to the present study, Gul *et al.* (2007) reported the better ADC values in diets of *Labeo rohita* containing optimal dietary protein level.

Digestive enzymes

The digestive enzyme activity levels of climbing perch fed with varying levels of dietary crude protein are shown in Fig 2, 3 and 4. The protease and amylase activities exhibited significant difference ($P<0.05$) across the treatments, while lipase activity did not show any significant difference among the treatments. The significantly higher protease activity was observed in the intestine (4.10 ± 0.07 U mg protein⁻¹ min⁻¹) and liver (2.52 ± 0.04 U mg protein⁻¹ min⁻¹) of the CP 38 dietary group. However, these results are not significantly varied with the 35% crude protein diet fed group. The highest amylase activity, in both the intestine (1.45 ± 0.05 U mg protein⁻¹ min⁻¹) and liver (0.81 ± 0.03 U mg protein⁻¹ min⁻¹), was observed in the CP 25 dietary group.

The digestive enzyme activities influence the nutrient utilization and fish growth performance which indicates the optimal availability of dietary nutrients in intestine

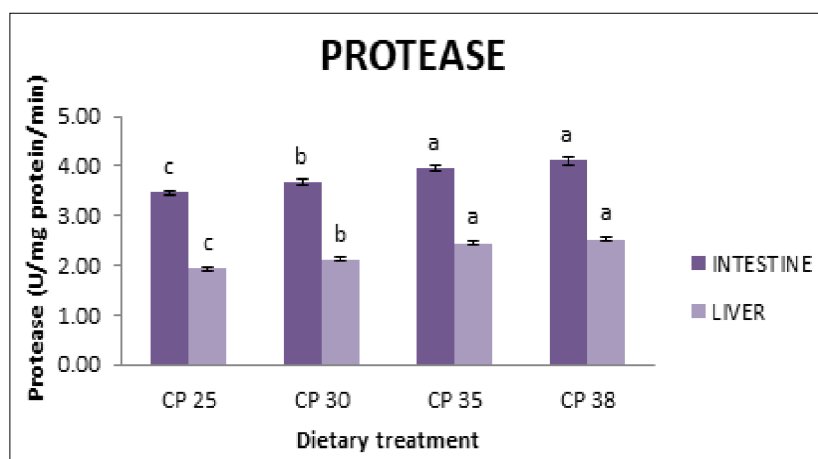


Fig 2: Protease enzyme activity of climbing perch fed with varying levels of dietary crude protein

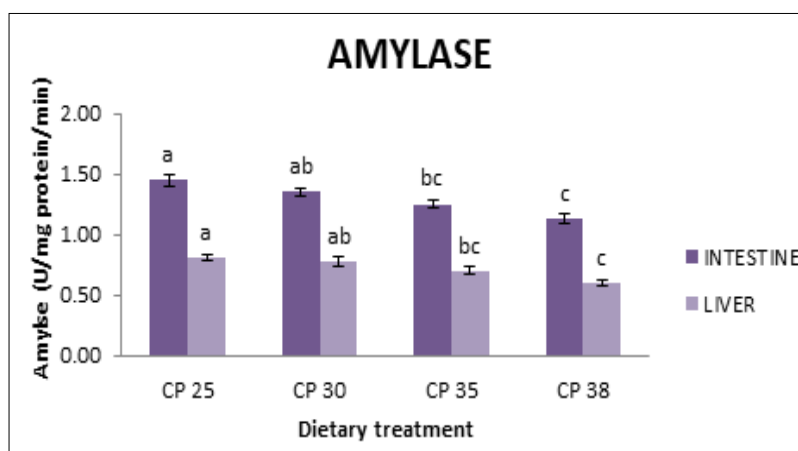


Fig 3: Amylase enzyme activity of climbing perch fed with varying levels of dietary crude protein.

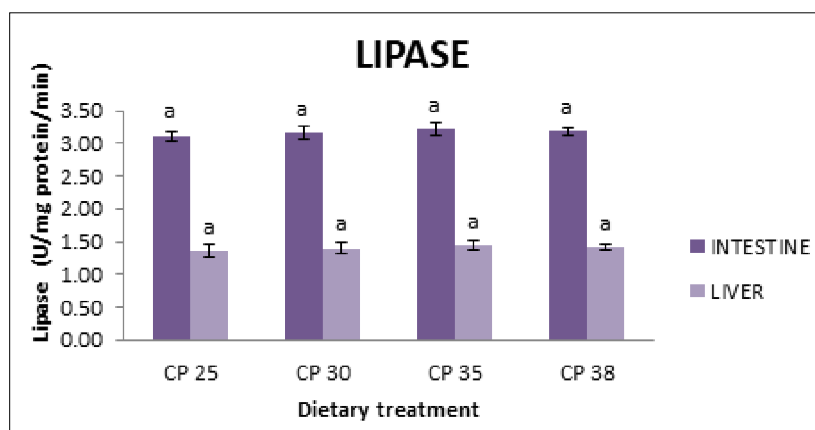


Fig 4: Lipase enzyme activity of climbing perch fed with varying levels of dietary crude protein.

Table 3: Whole body composition of climbing perch fed with experimental diets containing varying levels of dietary protein for 60 days.

Parameters	Initial	CP 25	CP 30	CP 35	CP 38	P value
Moisture (%)	80.32±0.12	77.99±0.07 ^a	77.24±0.06 ^b	76.76±0.04 ^c	76.60±0.05 ^c	<0.001
Crude protein (%)	12.32±0.08	13.61±0.04 ^c	13.97±0.05 ^b	14.36±0.06 ^a	14.48±0.04 ^a	<0.001
Crude lipid (%)	2.43±0.06	3.12±0.05 ^c	3.26±0.05 ^b	3.42±0.03 ^a	3.52±0.03 ^a	0.001
Crude fibre (%)	1.03±0.05	1.27±0.05 ^c	1.48±0.04 ^b	1.73±0.06 ^a	1.87±0.05 ^a	<0.001
Ash (%)	1.33±0.06	1.94±0.03 ^a	1.74±0.03 ^b	1.63±0.03 ^{bc}	1.61±0.04 ^c	0.001
Nitrogen free extract (%)	2.58±0.09	2.38±0.07 ^a	2.32±0.11 ^{ab}	1.83±0.11 ^{bc}	1.59±0.16 ^c	0.003

Values are presented as Mean ±SE. Means with different superscripts in a row differ significantly (P<0.05).

(Sagada *et al.*, 2017). Our results showed that dietary protein levels significantly influenced the digestive enzyme activities in climbing perch. Specifically, amylase activity in both the intestine and liver were inversely correlated with dietary protein content, while protease activity was enhanced with increasing protein levels. The amylase activity was decreased with increased levels of dietary protein, mainly due to the lower digestible carbohydrate (NFE) in the groups fed with higher protein diets (Mohanta *et al.*, 2008; Talukdar *et al.*, 2020, 2021). In contrast to the present results, amylase activity was increased in rainbow trout (*Oncorhynchus mykiss*) fed with higher protein diets (Kawai and Ikeda, 1973). On the other side, the protease activity was positively correlated with increased levels of dietary protein. These results consistent with the findings of Talukdar *et al.* (2020) in *Mugil cephalus*. Interestingly, lipase activity remained unaffected by varying levels of dietary protein, which could be attributed to the equivalent lipid content of the diets. Similar findings also reported by Lundstedt *et al.* (2004) in *Pseudoplatystoma corruscans* and Talukdar *et al.* (2021) in *Anabas testudineus*.

Whole body composition

Whole body compositions of climbing perch fed with varying levels of dietary crude protein are presented in Table 3. Significant difference (P<0.05) was observed in whole body composition of fish fed with different diets. The highest moisture content was recorded in CP 25 (77.99±0.07%) dietary group. The highest crude protein (14.48±0.04%), crude lipid (3.52±0.03%) and crude fibre (1.87±0.05%) was

observed in the CP 38 dietary group. In contrast, the ash content reduced with increasing levels of dietary protein, with the highest value was observed in CP 25 (1.94±0.03%) while the lowest was observed in CP 38 (1.61±0.03%). However, the study found increase in the crude protein, crude lipid and crude fiber contents at the end of the experiment.

Body composition of fish is influenced by various factors including dietary protein, digestible energy to protein ratio, size of the fish and environmental factors (Li *et al.*, 2006). In the present study, fish moisture content was linearly decreased with increasing levels of dietary crude protein in the diet. The minimum moisture content was recorded in the CP 38 group, while the highest protein content was noted in CP 38 group. These results indicated that whole body protein content of climbing perch increased with elevated levels of dietary protein. However, there were no significant differences in whole body composition between the fish fed the 38% (CP 38) and those fed with 35% (CP 35) crude protein diet. Similar results were also reported in *Paralichthys olivaceus* (Kim *et al.*, 2002), *Bidyanus bidyanus* (Yang *et al.* 2002), *Oncorhynchus mykiss* (Ahmed and Ahmad, 2020). Kim and Lee (2009) found that whole body protein content increased in a dose dependent manner in response to dietary protein levels and exhibited higher crude protein content on the dietary protein level where maximum growth rate was achieved. Crude lipid content increased with elevated dietary protein levels, which is in consistent with the findings of Khan *et al.* (1993) in *Mystus nemurus* and Siddiqui and Khan (2009)

in *Heteropneustes fossilis*. Crude fibre also increased with elevated levels of dietary protein. In contrast, ash content showed a negative correlation with the dietary protein levels.

CONCLUSION

The study results suggest that varying levels of dietary crude protein had a significant effect on growth performance, feed utilization, digestive performance and whole body composition of climbing perch, *Anabas testudineus*. The better growth performance was observed in fish fed with 35% and 38% crude protein fed groups. Therefore, 35% crude protein is considered as optimal for climbing perch fry due to its cost effectiveness, efficient nutrient utilization and reduced environmental impact.

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Conflict of interest

The authors declare that they have no conflict of interest.

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