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Effect of Dietary Protein Levels on Growth Performance, Whole-Body Composition and Digestive Enzyme Activity of Bronze Featherback, *Notopterus notopterus*

P. Yuvarajan[1,6]*, P. Chidambaram[2], B. Ahilan[3], A. Kathirvelpandian[4], E. Suresh[5], Mahadevi[6], R. Somu Sunder Lingam[7] and H. Manimaran[1]

[1]*Department of Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University (TNJFU), Dr. M.G.R Fisheries College and Research Institute (Dr. M.G.R FC and RI), Ponneri, Thiruvallur, Tamil Nadu, India*
[2]*TNJFU, Nagapattinam, Tamil Nadu, India*
[3]*TNJFU - Fisheries College and Research Institute, Thoothukudi, Tamil Nadu, India*
[4]*Peninsular and Marine Fish Genetic Resources Centre, ICAR-National Bureau of Fish Genetic Resources, CMFRI Campus, Kochi, Kerala, India*
[5]*Fish Genetics and Breeding Division, IFPGS, TNJFU, Vaniyanchavadi, Chennai, Tamil Nadu, India*
[6]*Department of Aquaculture, TNJFU - Dr. M.G.R FC and RI, Thalainayeru, Nagapattinam, Tamil Nadu, India*
[7]*TNJFU-DSA-Krishnagiri Barur Centre for Sustainable Aquaculture, Krishnagiri, Tamil Nadu, India*

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PY: Conducted the experiment, data analysis, interpretation and prepared original manuscript. PC: Conceptualized, designed and major supervision of the study. BA, AK and ES: Supervision and guidance of the study. Mahadevi and RSSL: Reviewed and edited the manuscript. HM: Assistance during experimental trial.

Key words
Digestive enzyme activity, Bronze Featherback, Growth performance, Nutrition, Protein requirement, Whole-body composition

ABSTRACT

The bronze featherback (*Notopterus notopterus*) is prioritized as a multi-potential candidate for the species diversification of aquaculture in Asia. To best of our knowledge, limited studies only attempted on this species' nutritional requirement. Hence, the present study was conducted to investigate the optimal protein requirement for the growth performance of fish. In this experiment, 240 fingerlings (initial average body weight, 20.38 ± 0.39 g fish^{-1}) were randomly distributed into four groups (T1, T2, T3 and T4), in triplicate, and were fed with 30, 35, 40 and 45 % crude protein (CP) with iso-lipidic diets, respectively for the period of two-months. In the current study, T3 diet (40% CP) fed fish showed significantly ($p < 0.05$) higher wight gain (WG), specific growth rate (SGR) and protein efficiency ratio (PER) compared to other dietary treatments. The whole-body CP was gradually increased with dietary protein elevation and found maximum at T3 diet fed fish then decreased in T4. Fish fed with high protein diet (T4 and T3) exhibited significantly ($p<0.05$) lower amylase and lipase activity compared to those on low dietary protein treatments (T1 and T2). On the other hand, protease activity was significantly ($p<0.05$) higher in the T3 diet fed fish than T4, T2 and T1. Overall, the present study suggests that, T3 (40% CP) diet enhances the feed utilization and growth performance of advanced fingerlings of *Notopterus notopterus*.

* Corresponding author: yuvarajandono@gmail.com
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INTRODUCTION

Species diversification facilitates the growth of aquaculture through extend its farming efficiency and market base (Cai *et al*., 2023). In India, several species have been prioritized for diversification and increase the production of freshwater aquaculture based on their culture potential and conservation. Bronze featherback (*Notopterus notopterus*), is one among the species (Ghatge *et al*., 2018) belonging to Osteoglossiformes, Notopteridae, is widely distributed in the South East

Asian Countries from Pakistan to Vietnam (Wibowo *et al.*, 2024). Despite the fact that it is least concern fish (Ng, 2021), versatility of this species will perform immense role in food, ornamental and sport fisheries sector. It is a carnivorous fish which widely feeds on crustaceans, insects, small fishes and aquatic plants (Gupta and Ray, 2022). Few studies have been conducted on various facets to domesticate this species in captive condition and commercialization (Rahmatullah *et al.*, 2009; Sukendi *et al.*, 2020; Muslim *et al.*, 2023; Yuvarajan *et al.*, 2024). However, a successful culture of any novel species hinges not only on their adaptability to captive environment but also their nutritional requirement for the optimal growth and survival. Among the nutritional factors, protein plays inevitable role in the physiological process of fish which govern growth, survival, reproduction and health.

Proteins are indispensable macronutrients comprised of amino acids, serving as the building blocks for various tissues, hormones, enzymes, and immune responses of fish. The protein utilization of fish is profoundly influenced by life stage, dietary preferences, digestive enzyme activity, metabolism, growth rate and culture system (Langi *et al.*, 2024). On the other hand, absorption of protein is lower than the physiological needs which cause negative impact on growth performance and immunity (Yan *et al.*, 2021), whereas, excess protein, increases the feed cost, deteriorates the water quality and retard the growth (Qian *et al.*, 2022). Consequently, understanding the species-specific protein requirement is imperative for the formulating diets that promote efficient growth and maximize the production yield. To the best of our knowledge, there is no study on the protein requirement of bronze featherback. Hence, the current study intended to optimize the dietary protein level on growth, survival and digestive enzyme activity of *Notopterus notopterus*.

MATERIALS AND METHODS

Experimental design

A 60-day feeding experiment was carried out at Anguraj Fish Farm, Ootamalai, Hogenakkal, Tamil Nadu, India, using hapas measuring 2 x 2 x 1 meters within an earthen pond. The study encompassed four different treatments (T1: 30% CP, T2: 35% CP, T3: 40% CP and T4: 45% CP) in triplicate.

Experimental fish

The necessary amount of bronze featherback fingerlings was sourced from the wild, River Cauveri, associated with Dharmapuri to Salem District and subsequently released into a hapa measuring 10 x 3 x 1 meter at the experimental site for acclimatization. Over the 15 days, the fish were acclimatized and nourished with a commercial diet (32% CP). Before the commencement of an experiment, the fish were graded, with approximately 240 fingerlings (20.38 ± 0.39 g weight fish^{-1}) were being randomly selected and distributed across four treatment groups, triplicate.

Experimental diets

Four experimental diets (T1, T2, T3 and T4) were formulated using fish meal, shrimp head meal, silkworm pupae and soy flour as protein sources (Table I) and prepared to contain iso-lipidic and graded levels of crude protein 30, 35, 40 and 45% of CP, respectively. All dry ingredients were pulverized into fine powder, weighed, steamed and subjected to a pelletizer with 3 mm die for the preparation of each diet. Then it was dried, crushed, packed, labelled and kept in separate container for the feeding trial. Bronze featherback fed with experimental diets, twice a day, at 9:00 AM and 4.00 PM.

Table I. Experimental diet formulation and proximate composition (%, dry weight) for bronze featherback, *Notopterus notopterus*.

Feed ingredients	T1 (CP 30%)	T2 (CP 35%)	T3 (CP 40%)	T4 (CP 45%)
Fish meal[a]	15	25	35	45
Silkworm pupae[b]	5	5	5	5
Shrimp head meal[a]	10	10	10	10
Soy flour[c]	27	27	27	27
Corn flour[c]	40	30	20	10
Fish oil[a]	2	2	2	2
Vitamin mineral premix[d]	1	1	1	1
Total	100	100	100	100
Proximate composition				
Moisture	10.12	10.24	10.36	10.48
CP[e]	30.17	35.71	40.25	44.79
C[f]	6.82	6.97	7.25	7.52
CF[g]	3.67	3.59	3.5	3.41
Ash	11.81	15.16	18.52	21.88

[a], K.S.S fish meal and dry fish, Thiruvallur, Tamil Nadu, India. [b], Silver Mines Silk Processors Private Limited, Udumalpet, Tamil Nadu, India. [c], Local market, Thiruvallur, Tamil Nadu, India. [d], Each Kg contains Vitamin A 7,00,000 IU, Vitamin D3 70000, Vitamin E 250 mg, Nictinamide 1000 mg, Cobalt 150 mg, Copper 1200 mg, Iodine 325 mg, Iron 1500 mg, Magnesium 6000 mg, Manganese 1500 mg, Potassium 100 mg, Selenium 10 mg, Zinc 19 mg, Calcium 26%, Phosphorus 13% (Indian Genome Private Limited, Hyderabad, India). [e], Crude protein; [f], Crude lipid; [g], Crude fibre.

Growth parameters

The growth performance of bronze featherback was monitored by measuring the length and weight. At the end of feeding trial, various growth parameters including weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), food conversion ratio (FCR), and protein efficiency ratio (PER) were determined using the following formulas:

WG (in g) = Final body weight (FBW, in g) – Initial body weight (IBW, in g)

DWG (in g) = FBW (in g) – IBW (in g)/ Experimental duration (in days)

SGR (%) = Ln FBW – Ln IBW/ Experimental duration (in days) ×100

FCR= Total feed given (in g)/ Total weight gain (in g)

PER = Body weight gain (in g)/ Protein intake (in g)

Whole-body proximate composition

Whole-body proximate composition of fish was estimated at the end of the experiment, following the standard procedures outlined by AOAC (1995). Crude protein, crude lipid and crude fibre were determined by kjeldahl method (utilizing Kjeltron Tulin equipments), soxhlet method (with SoxTRON SOX-2 Tulin Equipments) and analytical method (employing FibroTRON FRB-2 Tulin Equipments), respectively. The secondary moisture content of the fish was estimated using hot air oven and the total ash content was determined using a muffle furnase.

Intestinal digestive enzyme parameters

Three fish were chosen randomly from each hapa, and their intestine samples were aseptically extracted, pooled, and homogenized with a 0.25M sucrose solution. The resulting homogenate underwent centrifugation at 3070 x G for 5 min. Following centrifugation, the supernatant was utilized for enzyme assay. Amylase activity was measured spectrophotometrically at 560 nm using standard procedures (Bernfeld, 1955). Protease activity was analysed at 280 nm using the method described by Drapeau (1976). Lipase activity was assessed by measuring fatty acid release resulting from enzyme hydrolysis of olive oil, following the procedures outlined by Cherry and Crandell (1932).

Statistical analysis

All the data were presented as the mean values ± standard error of three replicates. The data were statistically analysed using one-way ANOVA by SPSS 25.0 version for windows followed by Duncan's test at the significant level of 0.05 was used to compare the differences among the four dietary groups.

RESULTS AND DISCUSSION

Growth performance and feed utilization

Bronze featherbacks' feed utilization and growth parameters are given in Table II. Treatments, T3 and T4 showed significantly ($p < 0.05$) higher FBW, WG, DWG, SGR, PER compared to T1 and T2. These parameters gradually increased in dietary treatment along with the augmentation of dietary protein level upto 40% Crude protein level then decreased (T4). This finding is similar with juvenile parrot fish *Oplegnathus fasciatus* (Kim *et al*., 2016) and large-size grouper, *Epinephelus coioides* (Yan *et al*. 2020), those authors obtained better growth performance upto certain level of dietary crude protein elevation, further increase of CP did not increase the growth. Because, excess dietary protein energy used for the regulation of nitrogen metabolism that might retard the fish growth (Qian *et al*., 2022). Fish fed with low-protein diet exhibited notably poor feed utilization, with highest FCR and lowest PER observed in T1 diet fed fish. At the same time, feed utilization increased according to the increment of dietary crude protein, with the optimal FCR and higher PER observed in fish fed T3 diet. However, FCR and PER showed a subsequent increase and decrease in high protein diet (T4), respectively.

Table II. Growth performance and feed utilization of Bronze featherback, *Notopterus notopterus* fed with different dietary protein.

Parameters	T1 (CP 30%)	T2 (CP 35%)	T3 (CP 40%)	T4 (CP 45%)
[1]IBW (g fish^{-1})	20.51 ± 0.33 ^{a}	20.24 ± 0.46 ^{a}	20.23 ± 0.44 ^{a}	20.52 ± 0.31 ^{a}
[2]FBW (g fish^{-1})	24.42 ± 0.43 ^{b}	25.07 ± 0.43 ^{b}	29.09 ± 0.06 ^{a}	28.68 ± 0.23 ^{a}
[3]WG (g fish^{-1})	3.92 ± 0.18 ^{b}	4.83 ± 0.36 ^{b}	8.86 ± 0.44 ^{a}	8.16 ± 0.23 ^{a}
[4]DWG (g fish^{-1})	0.07 ± .01 ^{b}	0.08 ± 0.01 ^{b}	0.15 ± 0.01 ^{a}	0.14 ± 0.01 ^{a}
[5]SGR (%)	0.29 ± 0.01 ^{b}	0.36 ± 0.03 ^{b}	0.61 ± 0.04 ^{a}	0.56 ± 0.02 ^{a}
[6]FCR	4.73 ± 0.20 ^{a}	3.82 ± 0.35 ^{b}	2.07 ± 0.14 ^{c}	2.27 ± 0.09 ^{c}
[7]PER	0.71 ± 0.03 ^{c}	0.76 ± 0.06 ^{c}	1.22 ± 0.09 ^{a}	0.98 ± 0.04 ^{b}

[1]Initial body weight; [2]Final body weight; [3]Weight gain; [4]Daily weight gain; [5]Specific growth rate; [6]Food conversion ratio; [7]Protein efficiency ratio. Values are expressed as mean ± standard error. The values are in same row with different superscripts indicate significant difference ($p<0.05$).

Similar result was obtained by Liu *et al*. (2023) who testified five different crude protein (22.52–45.78%) diets in the juvenile dotted gizzard shad and found maximum weight gain and better FCR for the optimal protein requirement (31.75–33.82% CP). Lowest PER in T1 diet fed fish due to the insufficient availability of dietary protein for the metabolism and muscle buildup which resulted poor growth. Similarly, Ahmed and Ahmed (2020) had evaluated the growth performance of rainbow trout with various dietary protein (30-55% CP) and found low PER value and poor growth in the low protein diet (30% CP). In the current study, there was no mortality was noticed in all the treatments.

Whole-body proximate composition

The results of whole-body composition are shown in Table III. Moisture, Crude lipid and ash did not show any significant difference (p>0.05) among the dietary treatments. But there was significantly (p <0.05) higher crude protein content was noticed in T3 and T4 diet fed whole-body fish groups compared to T2 and T1. This is may be due to the iso-lipidic and different-nitrogenous diet might influence the whole-body composition of fish. In this study, the crude protein content was gradually increased with dietary protein elevation and found maximum at T3 diet fed fish then decreased in T4, which reflected in the weight gain and PER. Similarly, Yan *et al*. (2021) found significantly higher crude protein content and lower crude lipid content of whole-body par with elevation of dietary crude protein in the treatments. However, Liu *et al*. (2023) found no significant difference among the dietary treatments.

Table III. Whole-body proximate composition of Bronze featherback, *Notopterus notopterus* fed with different dietary protein.

Parameters (%)	T1 (CP 30%)	T2 (CP 35%)	T3 (CP 40%)	T4 (CP 45%)
Moisture	7.30 ± 0.62^{a}	7.24 ± 0.50^{a}	7.08 ± 0.92^{a}	7.20 ± 0.62^{a}
CP[1]	58.55 ± 0.47^{b}	59.52 ± 0.49^{b}	63.27 ± 0.62^{a}	63.17 ± 0.63^{a}
CL[2]	2.17 ± 0.18^{a}	1.99 ± 0.08^{a}	1.87 ± 0.04^{a}	1.84 ± 0.03^{a}
Total ash	30.60 ± 0.41^{a}	30.25 ± 0.71^{a}	30.19 ± 1.02^{a}	30.02 ± 0.99^{a}

[1]CP, crude protein; [2]CL, crude lipid; Values are expressed as mean ± standard error. The values are in same line with different superscript letter indicates significant difference at p<0.05.

Intestinal digestive enzyme activity

The digestive enzyme activities of bronze featherback are given in Figure 1. In general, the digestive enzyme activities vary among the species based on age, diet composition and environmental factors

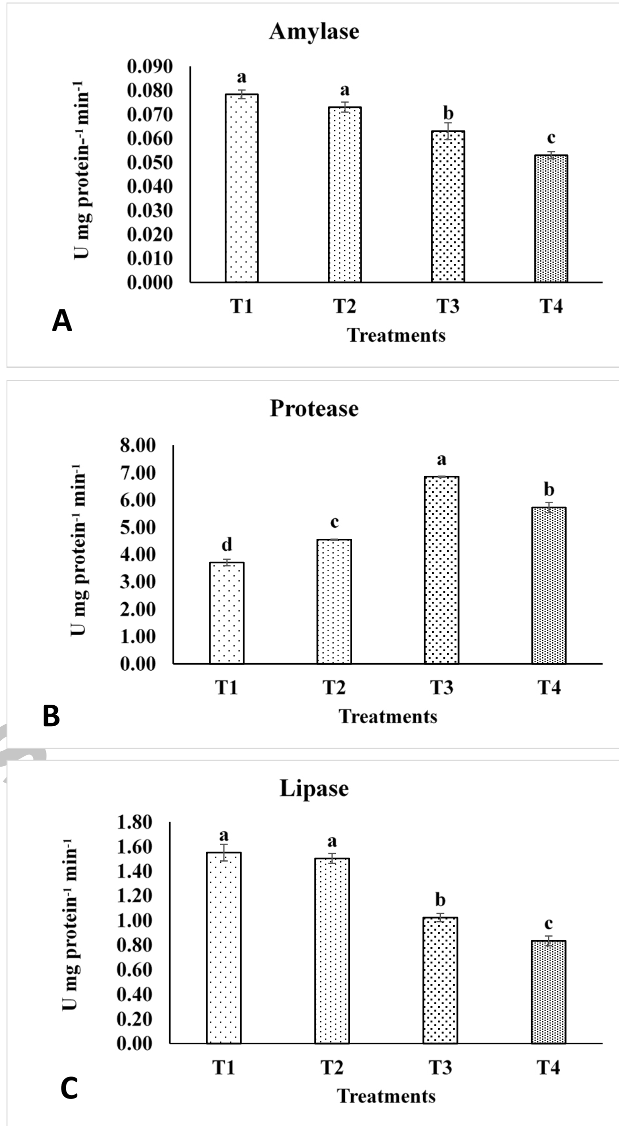

Fig. 1. Intestinal digestive enzyme (amylase, protease and lipase) activity in bronze featherback, *Notopterus notopterus* fed with different protein diets: T1 (30% CP), T2 (35% CP), T3 (40% CP) and T4 (45% CP). Values in the figures are means ± SE (n = 3); Different superscript letters indicate the significant difference (p<0.05).

(Riaz and Naeem, 2020). In the present study, significantly (p <0.05) lower amylase and lipase activity was noticed in the high protein diet fed fish (T4 and T3) compared to low dietary protein treatments (T1 and T2). Nazir *et al*. (2023) found the similar results in the Bullseye snakehead fed with different protein diet (40–55% CP) and found decreasing trend of amylase and lipase activity when the dietary protein increment in the fish diet. On the other hand, protease activity was significantly (p <0.05) higher in the

T3 diet fed fish than T4, T2 and T1. This may be due to the carnivorous fish exhibits poor carbohydrate utilization and needs high protein in their diet that results the lowest amylase activity and highest protease activity (Chakrabarti *et al*., 1995; García-Meilán *et al*., 2023). However, when fish is reached maximum protein utilization (T3) then the protease activity was dropped (T4). This may be due to the higher growth efficiency and PER in T3 than T4. This finding is consistent with Yan *et al*. (2021) who had studied the influence of different dietary protein on the digestive enzyme activity of orange-spotted grouper and found gradual increase of protease activity from 30 to 50% CP diet fed fish then it began to decrease in 55 and 60 % CP diet fed fish.

CONCLUSION

Bronze featherback reached maximum weight gain, specific growth rate and protein efficiency ratio in treatment 3, further increase of dietary crude protein level did not support the growth performance and feed utilization. Hence, the optimal protein requirement is 40% CP for the advanced fingerlings of *Notopterus notopterus*.

DECLARATIONS

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IRB approval

This research work has been approved by Advisory committee and Institutional Review Board of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Tamil Nadu, India

Ethical statement

The procedures of CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals), Ministry of Environment and Forests (Animal Welfare Division), Government of India was followed in this experiment.

Statement of conflict of interest

The authors have declared no conflict of interest.

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