



Optimization of Dietary Ingredients Composition for Bronze Featherback, *Notopterus notopterus*

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ABSTRACT

Species diversification plays crucial role to increase an aquaculture production through adoption of novel candidate species and standardization of cultivation technology. The present study aimed to optimize the dietary ingredients composition for Bronze featherback, *Notopterus notopterus*. A 60-day feeding trial was conducted to standardize the dietary preference of bronze featherback based on its growth performance, digestive enzyme activity and haematological responses. In this study, four different combination of feed ingredients such as T1 (Fishmeal), T2 (silkworm pupae), T3 (shrimp head meal) and T4 (mixture) used for experimental diet preparation. About 450 fingerlings (8.36 ± 0.03 g/fish) were randomly stocked in the four treatments and one control, triplicate and fed twice a day. Weight gain and specific growth rate were significantly ($p < 0.05$) increased in the T4 diet fed fish than T2, T3 and control, however no significant difference ($p > 0.05$) was observed between T4 and T1. Among the treatments, fish fed with T4 diet showed better haematological responses through an increment of white blood cell count, red blood count, haemoglobin and haematocrit value. Higher lipase activity was found in the T2 diet fed fish followed by T1, T4, T3 and control group fish. No significant difference ($p > 0.05$) was observed in the whole-body composition of fish among the treatments and control. The present study found T4 diet composition favours the growth performance of bronze featherback.

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Authors' Contribution

PY conducted experiments, sampling and data analysis, and wrote original draft. PC conceptualized, designed and supervision the study. RSSL did data analysis and interpretation of data, and drafting manuscript. Mahadevi and PC reviewed and edited the manuscript.

Key words

Bronze featherback nutrition, Growth performance, Haematological response, Dietary composition, *Notopterus notopterus*

INTRODUCTION

The world population is continuously increasing with food demand and malnutrition problem. In order to tide over the nutritional deficiency, aquaculture can be a possible food production enterprise which supplies a quality protein. In 2020, Finfish production contributes maximum share (65.71 %) in the global aquaculture production (FAO, 2022). Notwithstanding, the existing cultivable species alone do not fulfil the food demand, thus the species diversification is a viable solution to increase the aquaculture production (Jayasankar, 2018). Among the edible fish, bronze featherbacks is one of the candidate

species for aquaculture due to its delicacy and nutritive value (Kulkarni and Sudarshan, 2020).

Notopterus notopterus is locally called as serruppachi, seppili, seppatta, seppaala and paravaala in the various parts of Tamil Nadu, India. It is mainly found in stagnant turbid water bodies includes floodplains, ponds and reservoirs. It is a column feeder and carnivorous in nature which feeds on small fishes, insects, crustaceans, plants and organic matters (Srivastava *et al.*, 2012; Yanwirsal *et al.*, 2017). In general, the domestication of new species into the captive condition is facing a lot of glitches due to an insufficient knowledge over the biological, ecological and socio-economic factors, particularly, paucity of quality seed and feed (Fabrice, 2018; Teletchea, 2021). Hence, the species-specific feed designing can increase the performance of fish under captive condition (Shapawi *et al.*, 2014). A very limited studies only has been conducted on this species, *Notopterus notopterus* with reference to growth and survival (Sukendi *et al.*, 2020). There is no study has been attempted on the standardization of dietary composition for *Notopterus notopterus*. Hence, the present study is aimed to optimize the feed ingredients composition for bronze featherbacks on growth performance.

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MATERIALS AND METHODS

Experimental design

An experiment was conducted in hapas (2 x 2 x 1 m) fixed in earthen pond for the period of 60 days at Anguraj Private Fish Farm, Hogenakkal, Tamil Nadu, India. The study was designed with four treatments and one control, in triplicate.

Experimental fish

A required quantity of bronze featherback fingerlings were collected from the wild, River Kaveri basin (Biligundlu to Mettur) and stocked in the hapa (10 x 3 x 1m) at experimental site. Fishes were conditioned and fed with commercial diet for the period of one month. Prior to the experiment the fishes were graded and about 450 fingerlings (8.36 ± 0.03 g/fish) were selected and randomly stocked in the four treatments and one control, in triplicate and fed twice a day (0900 h and 1600 h).

Experimental diets

Four isonitrogenous (32% crude protein) experimental diets were prepared viz., T1 (+ Fishmeal (FM), - silkworm pupae (SWP), -shrimp head meal (SHM)), T2 (-FM, +SWP, -SHM), T3 (-FM, -SWP, +SHM), and T4 (+FM, +SWP, +SHM) and commercial diet was used as a control. The formulation of an experimental diet was given in Table I.

Table I. Experimental diet formulation for bronze featherback, *Notopterus notopterus*.

Feed ingredients	T1 (FM)	T2 (SWP)	T3 (SHM)	T4 (Mix)
Soy flour	35	35	35	35
Wheat flour	14.5	14.5	14.5	14.5
Fish meal (FM)	20	0	0	7
Silkworm pupae (SWP)	0	20	0	7
Shrimp head meal (SHM)	0	0	32	10
Corn flour	26.5	29	14.5	23.5
Fish oil	3	0.5	3	2
Min mix	0.5	0.5	0.5	0.5
Vitamin mix	0.5	0.5	0.5	0.5
Total	100	100	100	100
Proximate composition %				
Moisture	9.55	9.00	10.04	9.53
Crude protein (CP)	31.9	31.94	31.37	31.88
Crude lipid (CL)	6.11	7.16	6.07	6.35
Crude fibre (CF)	3.01	3.82	5.52	4.08
Ash	10.61	4.75	14.14	9.75

Growth parameters

Growth performance of bronze featherback was observed by sampling the length and weight of fish at every fortnight. At the end of the trial, growth parameters such as weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate (SR) were calculated by following equations:

$$\text{WG (g)} = \text{Final weight(g)} - \text{Initial weight(g)}$$

$$\text{DWG (g)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Experimental duration}}$$

$$\text{FCR} = \frac{\text{Dry feed fed (g)}}{\text{weight gain (g)}}$$

$$\text{SGR (\%)} = \frac{\text{Ln Final weight (g)} - \text{Ln Initial weight (g)}}{\text{Experimental duration} \times 100}$$

$$\text{PER} = \frac{\text{Body weight gain (g)}}{\text{protein intake (g)}}$$

$$\text{SR (\%)} = \frac{\text{Total number of fishes survived}}{\text{Total number of fishes stocked}} \times 100$$

Haematological and serum biochemical parameters

At the end of the experiment, three fish were randomly selected and blood sample was collected from the caudal vein of each fish by 1 ml syringe which was prewashed with 10% ethylene diamine tetraacetic acid (EDTA) to avoid the blood clot. Neubauer hematocytometer was used to test the red blood cell (RBC) counts and white blood cell (WBC). Cyanmethaemoglobin method was used to determine the haemoglobin (Hb) concentrations in the sample (Drabkin, 1946) and haematocrit (Ht) was determined by the microhematocrit method (Nelson and Morris, 1989). According to Wintrobe (1934) the erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were also calculated as follows:

$$\text{MCV (per } \mu\text{l)} = \frac{\text{Ht} \times 10}{\text{erythrocytes}}$$

$$\text{MCH (fl)} = \frac{\text{Hb} \times 10}{\text{erythrocytes}}$$

$$\text{MCHC (g/dl)} = \frac{\text{Hb} \times 100}{\text{Ht}}$$

On the other hand, non-EDTA coated tubes were used to clot the blood and kept for 2 h at 4°C for biochemical parameters analysis. After that blood serum was prepared by centrifugation (3500 x g) of clotted sample for 15 min at 4°C. Total protein, albumin, globulin, glucose, total cholesterol and triglycerides were analysed by automated biochemical analyser (ERBA, Mannheim, Germany).

Digestive enzyme parameters

Three fish were randomly selected from each hapa and intestine samples were aseptically removed, pooled and homogenized with 0.25 M chilled sucrose solution and, the homogenate was centrifuged at 3,070 x G for 5 min. After centrifugation, the supernatant was used for enzyme assay. Amylase activity was analysed in the spectrophotometer at

560 nm by following standard methods (Bernfeld, 1955). The method of Drapeau (1976) was used to analyze the protease activity at 280 nm. As per the Cherry and Crandell (1932), the lipase activity was analyzed by measuring fatty acid release caused by enzyme hydrolysis of olive oil. One unit of enzyme activity was expressed as 1 g of tyrosine, glucose, maltose and fatty acid released per minute.

Whole-body composition

At the end of the trial, whole body composition of fishes was estimated on dry weight-basis by following standard methods of AOAC (1995). Crude protein, crude lipid and crude fibre were determined by Kjeldahl method (Kjeltron Tulin equipments), soxhlet method (SoxTRON SOX-2 Tulin Equipments) and analytical method (FibroTRON FRB-2 Tulin Equipments), respectively. Hot air oven was used to estimate the secondary moisture content of fish. Ash was estimated by muffle furnace.

Statistical analysis

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

RESULTS AND DISCUSSION

Growth performance and feed utilization

Growth performance and feed utilization parameters of bronze featherbacks are given in Table II. Weight gain and specific growth rate were significantly ($p < 0.05$) increased in the T4 than T2, T3 and control diets fed fish. This may be due to the dietary preference and utilization of nutrient energy from the fish meal (7%), silkworm

pupae meal (7%) and shrimp head meal (10%). However, there was no significant difference ($p > 0.05$) was observed between T4 and T1, which is may be due to the higher inclusion (20%) of fish meal with basic ingredients in the T1 diet that would have been balanced the nutrient requirement of bronze featherback. Similarly, the better growth performance was noted by Sukendi *et al.* (2020) where the trash fish and mussel were used as a feed for bronze featherback. Food conversion ratio was found to be lower in the T4 followed by T1, T3, T2 and control which indicates the mixed diet (T4) showed the effective feed utilization. Calberg *et al.* (2015) testified the newly formulated test diet and found variations in the dietary preference of Arctic charr compared to control diet because of different ingredients composition was used for each diet. Among the dietary treatments and control, there was no mortality was noticed. This result is contrary to the findings of Sukendi *et al.* (2020) where the author noticed minor mortality during the experiment that may be due to use of unprocessed trash fish which deteriorates the water quality, however, there is no significant difference was observed.

Haematology and serum biochemical parameters

Fish fed with T4 diet showed a significantly ($p < 0.05$) higher Hb and Ht value compared to control and other dietary treatments, except T1 (Fig. 1). Kulkarni (2015) reported the haematology of wild caught bronze featherback in which Hb was lower (6.55 g/dl) than the current study (7.53–9.76 g/dl) but Ht value of fish showed contrary result compared to control and dietary treatment groups, except T4. This indicates T4 diet performance are par with natural dietary and immune response. WBC and RBC values were within an optimal range (Fig. 1) which was concurrence with the earlier report of Kavya *et al.* (2016)

Table II. Growth performance and feed utilization indices of bronze featherbacks.

Parameters	Control (n= 3)	T1 (n= 3)	T2 (n= 3)	T3 (n= 3)	T4 (n= 3)	p-value
IBW	8.36 \pm 0.03 ^a	8.36 \pm 0.03 ^a	8.36 \pm 0.03 ^a	8.36 \pm 0.03 ^a	8.36 \pm 0.03 ^a	1.000
FBW	10.63 \pm 0.51 ^b	13.20 \pm 0.56 ^a	11.46 \pm 0.26 ^b	11.33 \pm 0.44 ^b	14.33 \pm 0.60 ^a	0.001*
WG	2.27 \pm 0.53 ^b	4.83 \pm 0.57 ^a	3.10 \pm 0.30 ^b	2.96 \pm 0.44 ^b	5.96 \pm 0.59 ^a	0.001*
DWG	0.04 \pm 0.005 ^a	0.10 \pm 0.00 ^a	0.03 \pm 0.00 ^a	0.06 \pm 0.02 ^a	0.1 \pm 0.00 ^a	0.138
SGR	0.4 \pm 0.05 ^b	0.76 \pm 0.08 ^a	0.53 \pm 0.03 ^b	0.50 \pm 0.05 ^b	0.90 \pm 0.05 ^a	0.001*
FCR	2.96 \pm 0.14 ^a	2.00 \pm 0.26 ^{bc}	2.66 \pm 0.26 ^{ab}	2.20 \pm 0.30 ^{abc}	1.60 \pm 0.15 ^c	0.015*
PER	1.06 \pm 0.06 ^b	1.63 \pm 0.23 ^{ab}	1.20 \pm 0.11 ^b	1.50 \pm 0.20 ^{ab}	2.00 \pm 0.20 ^a	0.027*
Survival rate	100 \pm 0.0 ^a	100 \pm 0.0 ^a	100 \pm 0.0 ^a	100 \pm 0.0 ^a	100 \pm 0.0 ^a	-

IBW, initial body weight; FBW, final body weight; WG, weight gain; DWG, daily weight gain; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio. Values are expressed as mean \pm standard error. The values are in same line with different superscript letter indicates significant difference at $p < 0.05$. For details of treatment groups, see Table I.

and Kulkarni (2015). No significant ($p>0.05$) effect on MCH and MCHC values in the control and dietary treatment groups (Fig. 1). Similarly, Sudha *et al.* (2022) found no significant ($p>0.05$) effect on MCH and MCHC of striped catfish. However, the findings of present results were higher compared to earlier report of Kulkarni (2015). This may be due the influence of nutritional composition of various ingredients. Total protein, albumin, globulin and A/G ratio were higher in the T4 compared to control and other dietary treatments (Fig. 2); these results indicate the better immune response of bronze featherbacks (Fawole *et al.*, 2017). Fish fed with T2 diet reflected the increased serum glucose, total cholesterol and triglycerides (Fig. 2) due to the higher inclusion of silkworm pupae which

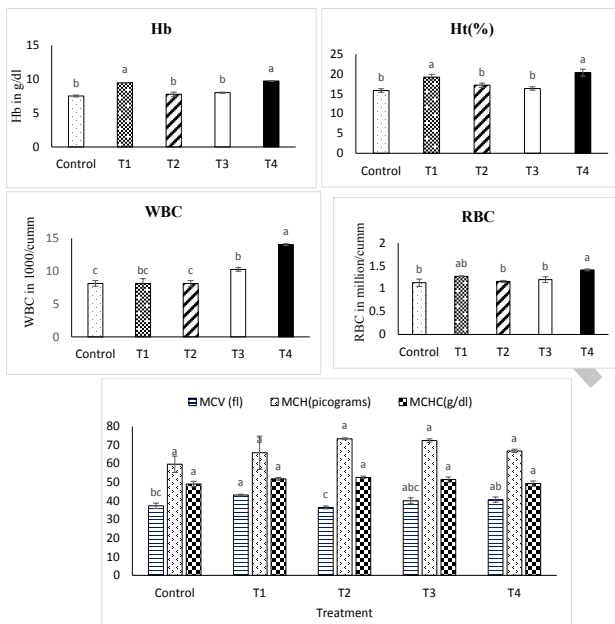


Fig. 1. Haematological response of Bronze featherback. The values are with different superscript letter indicates significant difference at $p<0.05$ for each parameter. For details of treatment groups, see Table I.

Hb, haemoglobin; Hct, haematocrit; RBC, red blood cell; WBC, white blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean cell haemoglobin concentration.

Digestive enzyme activity

The digestive enzyme activity results are presented in Fig. 3. Protease activity was significantly ($p<0.05$) higher in T4 compared to control and T2 whilst no significant ($p>0.05$) was observed among the T4, T1 and T3. This result was directly proportional to the PER results which indicates the utilization of proteins from the dietary ingredients with

the help of proteolytic bacteria (Vagharfard, 2020). The finding of present study was matching with the report of Sontakke *et al.* (2019) where the author found significantly higher protease activity in Chitala fed enriched artemia compared to control and unenriched moina. Captive rearing of bronze featherback had lower amylase activity as per the earlier report of Chakrabarti *et al.* (1995) in the wild *Notopterus notopterus*. In the current study, T4 showed the significantly higher ($p<0.05$) amylase activity than control however no significant difference ($p>0.05$) was noticed among the dietary treatment groups. Similar results were observed by Sen Gupta *et al.* (2021) who testified the

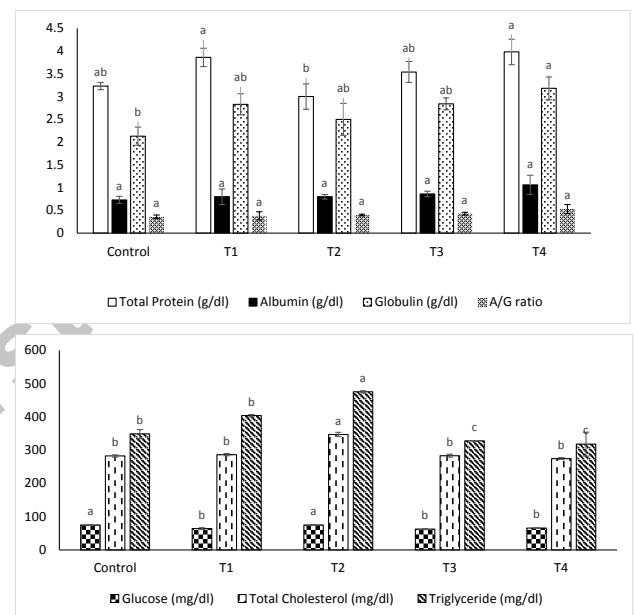


Fig. 2. Serum biochemical changes in bronze featherback. The values are with different superscript letter indicates significant difference at $p<0.05$ for each parameter. For details of treatment groups, see Table I.

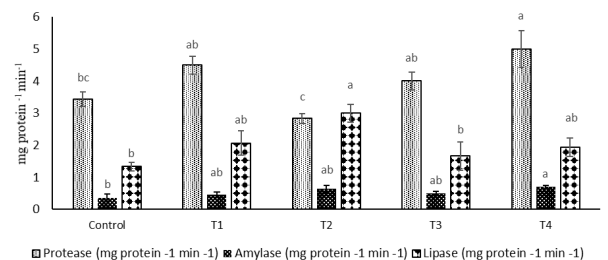


Fig. 3. Digestive enzyme activity of bronze featherback with response to different ingredients fed diets. The values are with different superscript letter indicates significant difference at $p<0.05$ for each parameter. For details of treatment groups, see Table I.

Table III. Proximate composition of whole-body composition (dry weight basis) of bronze featherback.

Parameters	Control	T1 (FM)	T2 (SWP)	T3 (SHM)	T4 (Mix)	p-value
CP	55.66 ± 1.45 ^{ab}	56.66 ± 0.88 ^a	53.0 ± 0.57 ^b	54.6 ± 0.88 ^{ab}	55.33 ± 0.88 ^{ab}	0.180
CL	9.33 ± 0.88 ^a	9.66 ± 1.20 ^a	11.0 ± 0.57 ^a	9.0 ± 0.57 ^a	9.33 ± 0.88 ^a	0.534
Ash	25.33 ± 1.76 ^a	22.0 ± 1.15 ^a	23.0 ± 1.15 ^a	23.33 ± 1.2 ^a	23.33 ± 0.88 ^a	0.491

Values are expressed as mean ± standard error. The values are in same line with different superscript letter indicates significant difference at $p < 0.05$. CP, crude protein; CL, crude lipid. For details of treatment groups, see Table I.

amylase activity in the wild caught *N. notopterus*. Higher lipase activity was found in T2 followed by T1, T4, T3 and control fish, respectively. Sontakke *et al.* (2019) found higher lipase activity in treatment compared to control due to enriched fatty acids, as like same, the maximum incorporation of silkworm pupae showed the higher lipase activity in the current study due to the high fat content in the SWP.

Whole-body composition

The whole-body composition of bronze featherback was given in Table III. No significant difference ($p > 0.05$) was observed in the whole-body composition of fish among the treatments and control (Table III). This is may be due to the isonitrogenous and iso energetic diet fed to all treatments. Similarly, Sathishkumar *et al.* (2021) found no difference in GIFT fed with SWP incorporated diet. However, T1 diet fed fish contains higher crude protein level in the whole-body compared to other dietary treatments and control which may be due to the effective utilization protein from fish meal diet. On the other hand, T2 diet fed fish showed higher deposition of crude lipid in their whole-body than other dietary treatment and control which may be the maximum inclusion of silkworm pupae in the T2 diet.

CONCLUSION

Fingerlings of bronze featherbacks fed with T4 diet showed the better growth performance, digestive enzyme activity and haematological responses. Moreover, the future research may be conducted to optimize the protein and lipid requirement of *Notopterus notopterus* to standardize the nutritional requirement of fish for better growth performance.

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IRB approval

The study was approved by Institutional Review Board of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Tamil Nadu, India.

Ethical statement

The experiment was conducted following the procedures of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forests (Animal Welfare Division), Government of India.

Statement of conflict of interest

The authors have declared no conflict of interest.

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