Influence of Varying Dietary Protein Levels and Partial Replacement of Fish Meal with Silkworm Pupae Meal on Growth Performance, Digestive Efficiency, Proximate Composition, and Sensory Attributes of Asian Stinging Catfish (*Heteropneustes fossilis*)

[1*]Abulhasan A, [2*]Aanand S, [3]Cheryl Antony, [4]Somu Sunder Lingam R

and [5]Karthireddy Syamala

[1*] Dr. M.G.R Fisheries College and Research Institute, Ponneri, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India- 601204

[2] Erode Bhavanisagar Centre for Sustainable Aquaculture (EBCeSA), Bhavanisagar, Erode, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India- 638 451

[3]Directorate of Sustainable Aquaculture, Soorakkottai, Tamil Nadu Dr J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India- 611 002

[4]Krishnagiri Barur Centre for Sustainable Aquaculture (KBCeSA), Barur, Krishnagiri, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Krishnagiri, Tamil Nadu, India – 635 201

[5]ICAR-Central Institute of Fisheries Education, Kakinada Centre, Kakinada, Andhra Pradesh, India- 533 001

Corresponding author information

*Corresponding author: Abulhasan A, Aanand

ABSTRACT

The present study was conducted to optimize the protein content in silkworm pupae-based diet for *Hetropneustes fossilis*. For this, the study developed six distinct experimental diets at different concentrations of dietary crude protein levels 32.5% (T_1), 35% (T_2), 37.5% (T_3), 40% (T_4), 42.5% (T_5), and 45% (T_6) and each diet incorporating a 50% substitution of fish meal with silkworm pupae meal. In the control diet, fishmeal has been kept without replacement with crude protein level of 40%. The experimental study carried out using 600 litres FRP tanks, each stocked with 40 stinging catfish fingerlings of size 0.466±0.01 g and 5.5±0.02 cm in triplicate and fed with 5% body weight with experimental diets. The significantly higher weight gain (7.13±0.12 g) and specific growth rate (4.66±0.02 %/day) was recorded in T_4 -40% dietary protein fed group fish. FCR was observed to be significantly lower in T_4 -40% (1.34 ± 0.03) and T_5-42.5% (1.40 ± 0.05) compared to other groups. Fish fed with T_4 -40% diet shown significantly higher FER (0.76±0.03) and PER (1.94±0.03). Intestinal protease activity was significantly lower in T_1 -32.5% (0.95±0.01) fed group, whereas lipase was significantly higher in T_6 -45% (2.68±0.20) and T_1 -32.5% (0.68±0.03) fed group shown higher amylase activity and significant difference was observed. T_4 -40% fed group shown significantly higher malate dehydrogenase (0.138±0.003) and lactate dehydrogenase (0.142±0.005) activity, whereas aspartate amino transferase (AST) and alanine amino transferase (ALT) doesn't show any significant differences. Hematological parameters such as RBC, WBC, Hct, Hb was found to be significantly differ among all the treatments and higher values were recorded in T_4 -40%, whereas mean corpuscular value (MCV) was significantly higher in T_1 -32.5% fed group. Whole body carcass composition of T_4

-40% fed diet shown significantly higher crude protein content, whereas crude lipid was found to be significantly higher in T_6 -45% fed group. On the basis of second-degree polynomial regression analysis, the study found a range of 40.45% - 41.20% is ideal crude protein for optimal growth of stinging catfish fingerlings. Overall, the present study recommends silkworm pupae at 50% replacement to fish meal in the diet of stinging catfish at a dietary protein level of 40% as ideal for better production performances.

Key words: Stinging catfish, Silkworm pupae meal, growth, enzyme activity

INTRODUCTION

Aquaculture provides much of the world's fish supply and is important in global food security. The global population growth is at a rate of 1.6 percent per year. In contrast, the aquaculture sector is expanding at 2.1 percent per year, outperforming other forms of animal production, such as livestock (Stankus 2021). Fish production must be increased to meet the world's rising protein needs. Aquaculture provides an important source of animal protein for human consumption. Fish develop rapidly and have a good calorie-to-protein ratio for human intake (Melenchón et al. 2022). Fish have also become a protein source that has proven to be twofold greater than poultry and threefold more than cattle (FAO 2010). The spectacular growth of the aquaculture industry is driven by fishmeal, a traditional protein source (Gasco et al. 2018). Fishmeal is considered an important protein source in feed due to its high digestibility, rich amino acid, and lipid composition (D'Agaro et al. 2022). This aquaculture industry growth has caused excessive fish meal demand (Alfiko et al. 2022) in already over-exploited capture fisheries. The overexploitation of oceans places unsustainable pressure on wild fish stocks, leading to their rapid decline. As a result, capture fisheries are projected to become unsustainable and economically unviable in the near future (Gasco et al. 2018). The rising cost and decreasing availability of fish meal further underscore the urgent need to minimize its use in feeds and explore more sustainable, cost-effective alternatives that maintain fish quality and nutritional value (Daniel 2018).

The scarcity and high cost of fishmeal have rendered fishmeal-based feeds a limiting factor in the aquaculture industry, driving the exploration of alternative protein sources with comparable nutritional value and high protein content. (Irm et al. 2022). Therefore, much research has been conducted to find an optimal ingredient for fishmeal by full or partial replacement (Singh et al., 2023). When choosing alternative feed components, the needs of the desired fish species need to be taken into account. Before deeming a new feed component adequate, factors like survival rates, growth potential, and feed conversion efficiency need to be assessed (Brown et al. 1996). The presence of anti-nutritional factors has limited the usage of plant-based ingredients in aqua feed formulation. To overcome these drawbacks, many strategies were deployed, such as the fermentation process and the usage of enzymes such as cellulase and phytase. Still, they lack the presence of critical amino acids such as lysine and methionine (Raghuvaran et al. 2024).

Silkworm pupae, a by-product of the silk reeling process, possess an impressive nutritional profile and have emerged as an alternative protein ingredient to fishmeal. While multiple species are utilized, *Bombyx mori* is the most prevalent. These pupae are byproducts of the silk industry and can be transformed into a high-quality, unconventional animal protein and energy-rich feed when processed appropriately. They also provide major essential nutrients such as protein, ether extract, crude fibre, nitrogen-free extract, ash, lysine, methionine, calcium and phosphorus (Habib and Hasan 1995). Utilizing affordable silkworm pupae as a substitute for fishmeal could

significantly lower the production costs of balanced fish diets, ultimately enhancing the profitability of fish farming. Its inclusion as a partial replacement to fish meal has performed well in many freshwater species, such as Rainbow Sharks (Raja et al. 2020), Rainbow Trout (Shakoori et al. 2016), Jian carp (Ji et al. 2015), Trout (Dheke and Gubhaju 2013), Deccan Mahseer (Shyama 1990), Chum Salmon (Akiyama et al. 1984). Silkworm pupae have also been observed to improve the growth and feed efficiency in many catfish species such as *Clarias batrachus* (Habib et al. 2001), *Clarias gariepinus* (Kurbanov et al. 2015a; Oso and Iwalaye 2014; Olaniyi and Babasanmi 2013).

Therefore, the present study investigated the effects of replacing FM protein with SWP meal with varying dietary protein levels on levels on growth, feed efficiency, and health condition of stinging catfish under captive conditions.

2. MATERIALS AND METHOD

2.1 Ethical statement

The study examined the optimization of dietary protein requirement of *H.fossilis* juveniles by partially replacing the silkworm pupae meal as an alternative protein source to the traditional fishmeal protein source.The present study adopted all the rules and regulations for experimental animal care and procedures stipulated by Tamil Nadu Dr J. Jayalalithaa Fisheries University (TNJFU), Nagapattinam, Tamil Nadu, India.

2.2 Experimental Setup

The experimental study was carried out at Erode Bhavanisagar Centre for Sustainable Aquaculture (EBCeSA), Bhavanisagar, Erode District, Tamil Nadu, India. The study was carried out in triplicates, in FRP tanks of 4.5 X 2.5 X 2.5 feet, each 600 liters capacity. Six Iso-nitrogenous diets with different crude protein levels 32.5%, 35%, 37.5%, 40%, 42.5%, and 45% designated as T_1, T_2, T_3, T_4, T_5, and T_6 each diet incorporating a 50% substitution of fish meal with silkworm pupae meal and the diet with only fishmeal has been kept as a reference diet (control) which has crude protein level of 40% (Table 1). Juvenile Asian stinging catfish (*H. fossilis*) fry, weighing approximately 0.466±0.01 g and 5.5±0.02 cm was stocked @ 40 in the experimental tanks. Feeding was done @ 5%/BW (Alam et al. 2009) twice daily at 9.00 am and 6.00 pm. Once in three days, 30% of the water was siphoned off and replaced with fresh water to maintain optimal conditions (Hossain et al. 2023).

2.3 Growth performance and feed utilization

At the end of the 80-day experimental study, all the fish were weighed individually by digital electric balance to analyse their weight gain. Growth performance and feed utilization were calculated using standard formulae (Zahan et al. 2024).

a) Mean weight gain (MWG) = Mean final weight (g) – Mean initial weight (g)
b) Specific growth rate (SGR) (%) = In final weight (g) – In initial weight (g) / Number of days X 100
c) Food conversion ratio (FCR) = Dry feed intake (g) / Weight gain (g)
d) Protein efficiency ratio (PER) = Weight gain (g) / Protein intake (g)
e) Survival rate (SR) (%) = (No. of fish survived/No. of fish stocked) × 100

After each sampling, the fish underwent a dip treatment with 0.1% potassium permanganate ($KMnO_4$) and a mild salt solution to alleviate physical stress and returned to their designated tanks.

2.4 Digestive enzyme assay

Five fish from each tank were euthanized at the end of the experiment, using anesthetic MS222 (2 g/l), and length and weight were measured. Fore gut samples were collected from the selected fish. The samples were individually homogenized using the Remi RQT 127 AQ homogenizer in an ice bath to prepare 5% tissue homogenate (tissue: homogenization buffer containing 0.02 M Tris /0.01 M phosphate, pH 7.0 in v/v glycerol), with a Teflon pestle of a motor-driven tissue-cell disrupter. The homogenates were centrifuged (Remi CPR 30 Plus) at 14000×g for 5 min, and the supernatants (crude homogenate) were used as the enzyme resource. The protein concentration in fish tissues was performed utilizing the method described by (Bradford 1976), with bovine serum albumin serving as a standard, The casein digestion method was adopted to assess the total proteolytic activity (Drapeau 1976). Amylase was determined by the Di-Nitro-Salicyclic acid (DNS) method (Rick and Stegbauer 1974). Lipase activity in the samples was determined using the titrimetric method by measuring the fatty acid release caused by enzyme hydrolysis of olive oil (Cherry and Crandall 1932), and the results expressed as (U/mg/protein).

2.5 Metabolic enzyme assay

Lactate dehydrogenase LDH activity was assayed following method of (Wroblewski and Ladue 1955). To start the reaction, sodium pyruvate is added to the reaction mixture at the pH of 7.5, NADH solution, 100 µl of tissue homogenate, and OD measured at 340 nm at 37 °C. The malate dehydrogenase MDH activity was assayed in tissues by the method of (Ochoa 1955). The MDH and LDH were expressed as (U/mg/protein). AST and ALT activity assays were carried out in the muscle tissue homogenates as described by (Wooten 1964), wherein α-ketoglutarate and dl-alanine were used as substrate for ALT and α-ketoglutarate and dl-aspartic acid were used as substrate for AST, OD measured at 540 nm, and the results were expressed as nm/mg/min.

2.6 Hematological parameters

At the end of the experimental trial, all the fish were fasted for 24 h and blood samples were collected from the caudal vein of three fish from each tank using a 1-ml syringe. The needle was dipped in 2% heparin solution as an anticoagulant during blood collection. Blood was then transferred into the tube containing EDTA and preserved at 4°C for hematological analyses (Neepa et al. 2022). The collected blood samples were poured into heparinized and non-heparinized tubes and stored immediately on ice. Red blood cell (RBC) and white blood cell (WBC) counts were determined using Neubauer hemocytometer. Haemoglobin (Hb) concentration was determined using cyanmethemoglobin method (Drabkin 1946) and haematocrit (Ht) was determined using microhematocrit method (Nelson et al. 1984). Erythrocyte indices such as Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were also calculated using the formula (Mrong et al. 2021; Kole et al. 2022) given below.,

MCV(fL)=Hct/RBC×10

MCH (pg)=Hb/RBC ×10

MCHC (%)=Hb/Hct×100

2.7 Statistical analysis

The data collected were processed and analysed by one-way ANOVA using SPSS version 27.0. In addition, multiple comparison analysis was carried out using Tukey's post-hoc with statistical significance for the test was set at $P<0.05$ between different groups. To predict more accurate responses to the dietary protein intake, the optimum level was estimated using second-degree polynomial regression analysis ($Y=aX^2+bX+c$) as described by **(Zeitoun et al. 1976).**

2.8 Proximate composition analysis

The proximate composition of experimental diets and fish, such as crude protein, lipid, moisture, and ash, were analysed in the laboratory of EBCeSA, TNJFU, Tamil Nadu, following protocols of(AOAC 2016). Crude protein was estimated using the Kjeldahl method; crude lipid was using the Soxhlet method with ether extraction; Moisture was determined by drying samples at 105°C to constant weight; Ash was performed by using a muffle furnace by incineration at 550 °C for 16 hrs. Dietary energy was analysed using the bomb calorimetry method.

2.9 Sensory evaluation of fish carcass

The sensory attributes assessed included appearance, taste, juiciness, and overall appearance of the flesh. These characteristics were evaluated using sensory techniques by a carefully chosen group of 10 panelists, Tastings took place in one session in a room. Tasters were familiarized with technical terms (odour, taste, acceptability) and then tasted cooked fish from various rations. A nine-point scale, was utilised for evaluation of the appearance. Moreover, a six-point scoring scale, was used for the assessment of the off-odour and off-flavour, respectively. An eight-point hedonic scoring scale was employed for juiciness and overall preferences.

2.9 Physicochemical parameters of water

Every two weeks, a comprehensive analysis of ten water quality parameters was conducted, including water temperature, pH, dissolved oxygen (DO), ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), inorganic phosphate (PO_4^-), potassium (K), hardness, alkalinity, total suspended solids (TSS) and total dissolved solids (TDS) (APHA 2012).

3. RESULTS

3.1 Growth performance and protein utilization

Heteropneustes fossilis readily consumed all the diets prepared for the experimental study. The experiment standardized the best dietary protein level of Silkworm pupae meal replaced with 50% of fishmeal for *H.fossilis*. The impacts of replacement of fishmeal on growth performance and protein utilization of stinging catfish juveniles are given in Table 2. The fish fed with a crude protein level of T4-40% obtained significantly higher ($P<0.05$) final body weight compared to other diets followed by T5-42.5% and T6-45% diet while the lowest was recorded in 32.5%. Specific growth rate (SGR), was significantly higher ($P<0.05$) in SWP followed by 42.5% and 45% crude protein diets. The best food conversion ratio (FCR) value of *H.fossilis* was obtained in T4 by feeding the fish 40% crude protein in diet, as it remained the significantly ($P<0.05$) lowest while the highest was recorded in T1 in which the fish supplied with 32.5% dietary protein level. Highest ($P<0.05$) value of protein efficiency ratio (PER) was recorded at the T4 in which the diet containing 40% protein level while the lowest was obtained at T1-

32.5%. Survival rate observed to be lower at T6 and higher at T4 where fish fed with 40% crude and a significant difference was occurred.

3.2 Digestive enzyme performance

The digestive enzyme activity, viz., protease, amylase, and lipase, varied significantly by including different diets in the experimental study and are shown in Table 3. The protease activity was higher in the treatment group T4 and significant difference ($P<0.05$) was observed. The T1 group where the fish fed with 32.5% dietary protein level obtained significantly higher amylase activity and decreased activity was observed beyond 40% dietary protein level in the diet. The activity of lipase was found to be significantly higher in T6 where the fish fed with 45% dietary protein level and lower in T1 where fish fed with 32.5% dietary protein.

3.3 Activities of Metabolic enzymes

The activity of metabolic enzymes malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) shown a significant difference ($P<0.05$) and their activity was higher in 40% dietary protein fed fish and declined beyond that level whereas lower level of activity was obtained at the treatment T1 where fish fed with 32.5% crude protein level in the diet. The AST activity of muscle tissue was highest in treatment T6, but did not differ significantly ($p>0.05$) from other treatments. Treatment T1 were fish fed with 32.5% crude protein shows higher activity of muscle tissue, in contrast it did not differ significantly ($p>0.05$) from other treatments and are table represented in Table 4.

3.4 Hematological indices

Hematological parameters of *H. fossilis* fed with different crude protein diet are presented in the Table 5. The fish fed with different diets shows a significant difference (($p>0.05$). The highest RBC concentration was exhibited by the diet T4 (40% CP), while lowest was in the treatment T1 (32.5%). WBC counts were highest in the fish fed with T4 diet and lower in T1. The fish fed wit treatment T4 exhibited highest HB and HCT concentration. The parameters such as MCV, MCHC obtained by treatment T4 were recorded higher but did not differ significantly from the treatment T5 and T6. MCH indices doesn't show any significant ($p>0.05$) between different treatments.

3.5 Statistical analysis

To generate statistically more precise information about the appropriate protein requirement, the growth data were subjected to second-degree polynomial regression analysis **(Fig. 1).** On subjecting a specific growth rate (SGR) data on (y-axis) and dietary protein levels on (X-axis), a break point was evident at 41.2 CP%. The relationship can be described by the following equation:

$$y= -0.0178x^2 + 1.14648x- 25.532 \ (R^2 = 0.8825)$$

On subjecting a Food Conversion Ratio (FCR) data on (y-axis) and dietary protein levels on (X-axis), a break point was evident at 40.45 CP%. The relationship can be described by the following equation:

$$y= 0.0035x^2 - 0.2973x + 7.7311 \ (R^2 = 0.8538)$$

3.6 Carcass composition

Table shows 6 significant variations (P<0.05) in fish body composition, including crude protein, crude lipid, ash, and moisture content. *H. fossilis* fingerlings fed 40% crude protein in the diet has highest crude protein content in the body. The crude lipid was highest in the treatment T6 followed by T5 and T4. All the treatment had substantial variations in moisture content, with the treatment T1 having the highest while T4 having the lowest. Ash was found to significantly higher in T1 fed diet while lower was observed at treatment T6.(Hervé et al. 2025)

3.7 Sensory attributes of fish

Most consumers found the appearance of the fish to be good across all diets (Table 7). The fish flesh was generally rated good for odour and taste except for the T6 diet where the fish fed with 45% crude protein level in the diet. The significant difference (P<0.05) was obtained at the sensory attributes for odour and taste of the fish. Overall, acceptability was high across all diets, with the highest percentage in treatment T4, where all panelists accepted the fish. The fish fed the T4 diet received unanimous appreciation from all panelists for its overall quality.

3.8 Water quality parameters

Different physio-chemical water quality parameters were analyzed and recorded fortnightly. Duplicate water samples were taken from each treatment, and the mean values recorded during the experimental period are given in Table 8. No significance (P>0.05) was observed in the water quality parameters during the experimental period.

4. DISCUSSION

Aquaculture sector faces a threat due to shortage of fish meal as it's the major component of a nutritionally good feed, which can support the growth and also fulfils the nutritional requirement of the fish (Rana et al. 2009). Hence, to bring about sustainability and to reduce the usage of fish meal in aquafeed, the current study aims to evaluate the partial replacement of fish meal by insect meal in Asian stinging catfish, *Heteropneustes fossilis*. Throughout the experimental study, fish that received diets with different levels of fishmeal protein substitution showed no apparent signs of nutritional deficiency. Water quality parameters were within the optimum range during the experimental period of 80 Days (Rahman et al. 2017).

The suboptimal growth rate observed at reduced protein intake levels may be attributed to the fish inability to effectively utilize dietary protein once it has attained the ideal protein threshold (Phillips 1972). The growth was found to be decreased beyond 40% in the treatment T5-42.5% and T6-45% as the dietary protein level increased in the diet. In both black catfish *Rhamdia quelen* (Salhi et al. 2004) and hybrid clarias, (*Clarias batrachus* × *Clarias gariepinus*, (Giri et al. 2003) dietary protein exceeding 40% led to lower growth performance indices. This can occur because the energy required for breaking down the excess protein is diverted away from the essential process of building muscle and tissue. The excessive amount of protein in the diet could diminish the performance of fish due to the process of catabolism where there will be a high demand for energy required than for protein deposition. The reduction in weight gain at high level of protein in the diet may also be due to inadequate non protein energy necessary to deaminate the high protein in the diet (Jauncey 1982)**.** The decrease in growth rate and decline in protein utilization outside requirement of dietary protein level is well recognized by various workers (Jobling and Wandsvik 1983**;** (Tibbetts et al. 2005)**;** Sa et al. 2006**;** (Kim and Lee 2005)**.** Ahmed and Ahmad (2020) observed similar results in *onchorhyncus mykiss* where growth performance was reduced once optimal protein was achieved.

The FCR, FER, PER was also significantly higher in T4-40% fed group and tends to be decreased and the results were similar to the findings of (Siddiqui and Khan 2009)**.** However, a significant fall in growth and conversion efficiencies was noted at 42.5% and 45% protein of the diet indicating that 40% protein diet (T4) satisfied the requirement and is considered optimum for achieving maximum growth and excellent conversion efficiency at 50% replacement of fishmeal by silkworm pupae meal. This outcome was in line with findings of (Kurbanov et al. 2015b)**,** reported that *Clarias gariepinus* fingerlings performed better at 50% replacement of fishmeal by silkworm pupae meal at a dietary protein level at 40% CP. Alongside these findings, many studies have shown that higher protein in the diet not only resulted in poor or stunted growth but also led to significant feed wastage, making it as an unwise economic choice (Ye et al. 2017)**.** From the second-degree polynomial regression analysis of the growth and body composition data, the optimum dietary protein level for growth of young *H. fossilis* is found to be in the range of 40.45%- 41.2% CP.

The whole-body carcass composition of Asian Stinging catfish fingerlings was significantly ($P<0.05$) affected by varying level of dietary protein in the diet. The crude protein content in the body was increased with dietary protein level, and the higher percentage of crude protein was observed in the 40% diet, which is in line with the outcome of (Khan et al. 1993; Ahmed and Ahmad 2020; Hossain et al. 2023).In contrast, no variation was reported by some studies as increase in dietary protein levels in the diet (Winfree and Stickney 1981)**.** The fat content in the body gradually increased as the dietary protein level increased in the diet with maximum lipid content was observed at highest protein containing diet 45%CP. This may be attributed to the factor that excess protein in the diet leads to deamination, which results in the deposition of excess protein as body fat (Deng et al. 2011) or it may be utilized for the metabolism as energy fuel (Wu and Gatlin III 2014). The moisture decreased with increase in dietary protein level in the body upto 40% dietary protein in the diet and tends to be increased beyond that level. Similar results were reported by various works (Jayant et al. 2018; Ahmed and Ahmad 2020). The elevated ash content observed at marginal protein levels could be attributed to the reduction in fish weight at lower protein concentrations, which ultimately impacts muscle development. Additionally, diets with lower protein content have also been linked to muscle hypertrophy (Yamashiro et al. 2016).

Blood parameters analysis is recognized as an essential method for assessing how different ingredient replacement in the diet of fish impacts its health status (Fagbenro et al. 2013; Shamna et al. 2017). In this RBC count showed an elevation with dietary protein levels up to 40%CP, and again a decrease trend was observed. The elevation may be due to the early release from the storage pool in the spleen (Vijayan and Leatherland 1989) and a decline have occurred in low diet may be due to the less oxygen supply to the cells of a lower protein fed group, which leads to reduction in growth (Silva et al. 2012). Similarly, Hct and Hb value increased with dietary protein level upto 40%, which indicates the well transport of oxygen in the body, leading to improved growth and fish health (Ahmed and Ahmad 2020). On the other hand, WBC count remains to be unchanged among different dietary protein fed groups in the study which is similar to the findings of (Debroy et al. 2024). The Hb content increased as dietary protein increased up to 40% and a decline was observed at 42.5% and 50%. While the poorest Hb content was at lowest level of protein diet 32.5%, these findings similar to the study in (Ahmed and Maqbool 2017). Hct value significantly higher at 45% and tends to decline beyond 40%, the findings were agreed with the results of (Ahmed and Ahmad 2020).

Intestinal protease activity was found to be higher in T4 fed group. The protease activity increased with increasing dietary protein level up to 40% and it starts to decline thereafter. Similar results were observed and reported by many researchers in silver barb, *Puntius gonionotus* (Mohanta et al. 2008), *Pangasianodon hypophthalmus* (Jayant et al. 2018), red tilapia juveniles *Oreochromis sp.* (Santos et al. 2020) and Striped Catfish *Pangasius hypophthalmus* (Bano et al. 2023). Amylase activity in the study was found to be in a decreasing trend as increase in dietary protein level. This result may be attributed to the factor that decline in dietary free extract by increasing crude protein level in the diet as a means to balance the feed formulation (Mohapatra et al. 2012). *O. niloticus* exhibits decreased amylase activity with increased dietary protein levels (Yang et al. 2018). The intestinal lipase activity of the fish tends to be increased as the protein level increased in the present study. In comparison to number of past studies, lipase activity decreased with an increase in the dietary protein level in the body. But this result positively agrees with the outcomes of (Ma et al. 2020), who reported the same trend of Lipase activity in the intestine increased significantly as dietary lipid level increased ($P<0.05$) in juvenile Small yellow croaker (*Larimichthys polyactis*). This study recorded the highest MDH and LDH activities at 40% protein. The elevated activity of MDH with increase in transaminase enzyme activitiy, coupled with higher protein in the diet indicates the availability for gluconeogenesis which can be further used for physiological activities of the fish. Similar increase in gluconeogenic activity was observed in black catfish *Rhamdia quelen* (Melo et al. 2006). Conversely the lower activity of MDH when fed with lower protein diet 20% was observed in *Pangasianodon hypophthalmus* (Jayant et al. 2023). Lower activity of MDH at low dietary protein fed group may be a sign of dimnished metabolic activity which may lead to a less availability of oxaloacetate as a substrate due to lower transaminase enzyme activities (Yengkokpam et al. 2013). The lower activity of LDH in lower protein fed diet may be due to the higher carbohydrate content which leads to metabolic stress(Kumar et al. 2009). The activity of ALT and AST of muscle were higher in 45% crude protein fed diet relatively higher compared to other diets. However, it does not significantly differ from 40% & 42.5% fed diet. Similar results were reported in *Pangasianodon hypophthalmus* (Tok et al. 2017). The elevation of ALT and AST while feeding higher dietary protein in the feed is may be attributed to the rise in transamination of the dietary protein (Pieper and Pfeffer 1980).

The diet fed with 45% dietary protein level showed a significant difference compared to other treatments, while a significant difference observed in odour, flavour, taste and in overall acceptance by the consumer. The difference would have been aroused due to higher deposition of lipid in the body of 45% dietary protein fed fishes. Diet is known to influence the organoleptic quality of fish (Spinelli et al. 1979). Earlier studies with Indian major carps have also shown no adverse effect of silkworm pupae on organoleptic quality (Jayaram and Shetty 1980; Nandeesha et al. 1990). Fat content of raw pupa is known to induce off-flavour (Hora 1962) and unpleasant taste(Rath 2018)when incorporated in the feed and the inclusion level rises, while in the present study as the dietary protein rises the inclusion level of silkworm pupae in the diet also increases, this can be attributed to the factor of off odour, flavour, taste and acceptance by consumers.

On the basis of second-degree polynomial regression analysis of growth parameters and body composition data revealed that, the optimum dietary protein level for best growth of stinging catfish fingerlings is recommended within the range from 40.8%-41.2%. The dietary protein requirement of stinging catfish fingerlings estimated during the present study is similar and the level fall in the range of previously reported dietary protein requirements

of some other fishes and catfish species such as young *H. fossilsi* 40-43% (Siddiqui and Khan 2009), *H.fossilis* 40.8-41.8% (Fatma and Ahmed 2020), walking catfish, *Cyprinus carpio* 41.25% (Ahmed and Maqbool 2017).

From the above experimental trial, results obtained fishes fed with T4- 40% dietary protein level have better performance concerning growth performance, general acceptability of meat quality, and improved digestive activity. Improved performance could be due to the better feed utilization and nutrient absorption. Beyond 40% dietary protein the growth tends to be declined According to (Council and Nutrition 1994), the protein requirements of various fish species are different. The protein requirements thus differ from one species to another. Some of the reasons why the protein requirements of fish vary have been attributed to include such factors such as different dietary formulas, size, and other research methods and analyses (Tibbetts et al. 2005). In addition, it might come in because of differences in laboratory settings, experimental design for example, feeding rate and frequency, water quality and flux, stocking density, and origin of protein used for each diet (Kim et al. 2001).

5. CONCLUSION

In conclusion, the silkworm pupae at 50% replacement of fish meal and at a dietary protein level of 40.45%-41.2% is ideal in the diet of stinging catfish for better performance with regard to growth, digestive enzyme activity, whole body carcass composition and meat quality.

6. CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

7. STATEMENT AND DECLARATIONS

The experiment was conducted at the Erode Bhavanisagar Centre for Sustainable Aquaculture (EBCeSA), Bhavanisagar, Erode District – 638451, Tamil Nadu, India. All procedures involving experimental animals were carried out in compliance with the guidelines for animal care and ethics established by Tamil Nadu Dr. J. Jayalalithaa Fisheries University (TNJFU).

The authors confirm that there are no financial conflicts of interest or personal associations that could have influenced the findings presented in this study.

8. AUTHOR CONTRIBUTIONS

Methodology, writing, original draft, Preparation, investigation, methodology, and formal analysis – Abulhasan A

Conceptualization, acquisition, resources, and supervision – Abulhasan A, Aanand S, Cheryl Antony, Somu Sunder Lingam R, and Karthireddy Syamala.

9. ACKNOWLEDGMENTS, FUNDING SOURCES

The author is thankful to the funders or the financial assistance for the research work fund provided by the Tamil Nadu Dr. J. Jayalalithaa Fisheries University (TNJFU), Nagapattinam, for the necessary support and facilities for carrying out the research programme. The authors expressed their gratitude to the Dean of Fisheries College and Research Institute, Thoothukudi, as well as the Assistant Professor and Head, Erode Centre for Sustainable Aquaculture (EBCeSA) for their indispensable support, encouragement, and provision of all necessary facilities, which were crucial for the successful completion of this research work.

10. DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are not publicly available due to institutional restrictions, but are available from the corresponding author on reasonable request due to confidential information.

References

Ahmed I, Ahmad I (2020) Effect of dietary protein levels on growth performance, hematological profile and biochemical composition of fingerlings rainbow trout, Oncorhynchus mykiss reared in Indian himalayan region. Aquaculture Reports 16:100268

Ahmed I, Maqbool A (2017) Effects of dietary protein levels on the growth, feed utilization and haemato-biochemical parameters of freshwater fish. Cyprinus Carpio Var Specularis :1–12

Akiyama T, Murai T, Hirasawa Y, Nose T (1984) Supplementation of various meals to fish meal diet for chum salmon fry. Aquaculture 37(3):217–222

Alam MJ, Mustafa MG, Khaleque MA (2009) Evaluations of the effects of different dietary vitamin C levels on the body composition, growth performance and feed utilization efficiencies in Stinging Catfish, Heteropneustes fossilis (Bloch, 1792). Journal of American Science 5(3):31–40

Alfiko Y, Xie D, Astuti RT, Wong J, Wang L (2022) Insects as a feed ingredient for fish culture: Status and trends. Aquaculture and Fisheries 7(2):166–178

AOAC GW (2016) Official methods of analysis of AOAC International. AOAC International, Rockville, MD, USA

APHA L (2012) Standard methods for the examination of water and wastewater. American public health association Washington, DC

Bano S, Khan N, Arslan M, Fatima M, Khalique A, Wan AH (2023) Impact of various dietary protein levels on the growth performance, nutrient profile, and digestive enzymes activities to provide an effective diet for striped catfish (Pangasius hypophthalmus). Turkish Journal of Fisheries and Aquatic Sciences 23(7)

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry 72(1–2):248–254

Brown PB, Dabrowski K, Garling DL (1996) Nutrition and feeding of yellow perch (Perca flavescens). J Appl Ichthyol 12(3–4):171–174. https://doi.org/10.1111/j.1439-0426.1996.tb00084.x

Cherry IS, Crandall LA (1932) THE SPECIFICITY OF PANCREATIC LIPASE: ITS APPEARANCE IN THE BLOOD AFTER PANCREATIC INJURY. American Journal of Physiology-Legacy Content 100(2):266–273. https://doi.org/10.1152/ajplegacy.1932.100.2.266

Council NR, Nutrition S on P (1994) Nutrient requirements of poultry: 1994. National Academies Press

D'Agaro E, Gibertoni P, Esposito S (2022) Recent trends and economic aspects in the rainbow trout (Oncorhynchus mykiss) sector. Applied Sciences 12(17):8773

Daniel N (2018) A review on replacing fish meal in aqua feeds using plant protein sources. International Journal of Fisheries and Aquatic Studies 6(2):164–179

Debroy S, Banerjee Sawant P, Sardar P, Biswas G, Varghese T, Goswami M, Kumar Maiti M, Haque R, Roy U, Sarma D (2024) Effects of Varying Dietary Protein Levels on Growth Performance, Survival, Body Composition, Haemato-Biochemical Profile, and Metabolic Responses of Hypselobarbus jerdoni (Day, 1870) Juveniles. Journal of Applied Ichthyology 2024:1–15. https://doi.org/10.1155/2024/8627712

Deng D-F, Ju ZY, Dominy W, Murashige R, Wilson RP (2011) Optimal dietary protein levels for juvenile Pacific threadfin (Polydactylus sexfilis) fed diets with two levels of lipid. Aquaculture 316(1–4):25–30

Dhar V, Singh SK, Narsale SA, Debbarma S, Saikia P, Yirang Y (2024) Fishmeal substitutions and their implications for aquatic animal immune and gut function: a review. Comparative Immunology Reports :200171

Dheke S, Gubhaju SR (2013) Growth response of rainbow trout (Oncorhynchus mykiss) on substitution of shrimp meal by different protein sources. Nepalese Journal of Zoology 1(1):24–29

Drabkin DL (1946) Spectrophotometric studies: XIV. The crystallographic and optical properties of the hemoglobin of man in comparison with those of other species. Journal of biological chemistry 164(2):703–723

Drapeau GR (1976) [38] Protease from Staphyloccus aureus. In: Methods in enzymology. Elsevier, pp 469–475

Fagbenro OA, Adeparusi EO, Jimoh WA (2013) Haematological profile of blood of African catfish (Clarias gariepinus, Burchell, 1822) fed sunflower and sesame meal based diets. Journal of Fisheries and Aquatic Science 8(1):80

FAO (2010) FAO. World Concern, Save the Children, ACDI/VOCA, Save the Children and World Vision

Fatma S, Ahmed I (2020) Effect of water temperature on protein requirement of Heteropneustes fossilis (Bloch) fry as determined by nutrient deposition, hemato-biochemical parameters and stress resistance response. Fish Aquatic Sci 23(1):1. https://doi.org/10.1186/s41240-020-0147-y

Gasco L, Gai F, Maricchiolo G, Genovese L, Ragonese S, Bottari T, Caruso G (2018) Fishmeal Alternative Protein Sources for Aquaculture Feeds. In: Feeds for the Aquaculture Sector. Springer International Publishing, Cham, pp 1–28

Giri SS, Sahoo SK, Sahu AK, Meher PK (2003) Effect of dietary protein level on growth, survival, feed utilisation and body composition of hybrid Clarias catfish (Clarias batrachus\times Clarias gariepinus). Animal Feed Science and Technology 104(1–4):169–178

Habib MAD, Hasan MR (1995) Evaluation of silkworm pupae as dietary protein source for Asian catfish, Clarias batrachus (L) fingerlings. Bangladesh Journal of Aquaculture 17:1–7

Hervé MK, Calice MD, Dzepe D, Djouaka RF, Chia SY, Efole T, Ndindeng SA (2025) Black soldier fly (Hermetia illucens) larvae improve growth performance and flesh quality of African catfish (Clarias gariepinus). Discov Anim 2(1):9. https://doi.org/10.1007/s44338-024-00045-8

Hora SL (1962) Handbook of fish culture in the Indopacific region. FAO Fish Biol Tech Pap 14:1–203

Hossain A, Hossain MdA, Rasul MdG, Akter T, Zaman MdFU, Islam MdR (2023) Efficacy of using sunflower meal as an ingredient, and partial fishmeal-replacer, in practical feed formulated for stinging catfish (*Heteropneustes fossilis*). Aquaculture Fish & Fisheries 3(3):237–246. https://doi.org/10.1002/aff2.109

Irm M, Ye B, Wu X, Geng L, Cai Q, Zhang L, Zhai H, Zhou Z (2022) Assessment of conventional and low gossypol cottonseed meal as alternative protein sources in low-fishmeal diets of hybrid grouper (Epinephelus fuscoguttatus♀\times Epinephelus lanceolatus♂): growth, feed utilization, gut histology, and immunity. Animals 12(15):1906

Jauncey K (1982) The effects of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapias (Sarotherodon mossambicus). Aquaculture 27(1):43–54

Jayant M, Muralidhar AP, Sahu NP, Jain KK, Pal AK, Srivastava PP (2018) Protein requirement of juvenile striped catfish, Pangasianodon hypophthalmus. Aquacult Int 26(1):375–389. https://doi.org/10.1007/s10499-017-0216-0

Jayant M, MURALIDHAR AP, SAHU NP, SRIVASTAVA PP, YADAV R, MEENA DK (2023) PHYSIO-METABOLIC AND HAEMATOLOGICAL CHANGES IN STRIPED CATFISH, PANGASIANODON HYPOPHTHALMUS FED WITH GRADED LEVEL OF PROTEIN. J Inland Fish Soc India 55(2):156–170

Jayaram MC, Shetty HPC (1980) Studies on the growth rates of catla, rohu and common carp fed on different formulated feeds.

Ji H, Zhang J-L, Huang J-Q, Cheng X-F, Liu C (2015) Effect of replacement of dietary fish meal with silkworm pupae meal on growth performance, body composition, intestinal protease activity and health status in juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquac Res 46(5):1209–1221. https://doi.org/10.1111/are.12276

Jobling M, Wandsvik A (1983) Quantitative protein requirements of Arctic charr, *Salvelinus alpinus* (L). Journal of Fish Biology 22(6):705–712. https://doi.org/10.1111/j.1095-8649.1983.tb04230.x

Khan MS, Ang KJ, Ambak MA, Saad CR (1993) Optimum dietary protein requirement of a Malaysian freshwater catfish, Mystus nemurus. Aquaculture 112(2–3):227–235

Kim JD, Lall SP, Milley JE (2001) Dietary protein requirements of juvenile haddock (*Melanogrammus aeglefinus* L.): Protein requirements of juvenile haddock. Aquaculture Research 32:1–7. https://doi.org/10.1046/j.1355-557x.2001.00001.x

Kim LO, Lee S-M (2005) Effects of the dietary protein and lipid levels on growth and body composition of bagrid catfish, Pseudobagrus fulvidraco. Aquaculture 243(1–4):323–329

Kole K, Islam MR, Mrong CE, Neepa NN, Sultana N, Haque MR, Salam S, Mostakim GM (2022) Toxicological effect of sumithion pesticide on the hematological parameters and its recovery pattern using probiotic in Barbonymus gonionotus. Toxicology Reports 9:230–237

Kumar A, Bhatnagar A, Garg SK (2009) Growth performance, carcass composition and digestive enzyme activity of pearlspot, Etroplus suratensis (Bloch) reared in inland saline groundwater ponds providing substrate or feed. Livestock Research for Rural Development 21(10):11–15

Kurbanov AR, Milusheva RY, Rashidova SS, Kamilov BG (2015a) Effect of replacement of fish meal with silkworm (Bombyx mori) pupa protein on the growth of Clarias gariepinus fingerling. Int J Fish Aquat Stud 2(6):25–27

Kurbanov AR, Milusheva RY, Rashidova SS, Kamilov BG (2015b) Effect of replacement of fish meal with silkworm (Bombyx mori) pupa protein on the growth of Clarias gariepinus fingerling. Int J Fish Aquat Stud 2(6):25–27

Melenchón F, de Mercado E, Pula HJ, Cardenete G, Barroso FG, Fabrikov D, Lourenço HM, Pessoa M-F, Lagos L, Weththasinghe P (2022) Fishmeal dietary replacement up to 50%: A comparative study of two insect meals for rainbow trout (Oncorhynchus mykiss). Animals 12(2):179

Melo JFB, Lundstedt LM, Metón I, Baanante IV, Moraes G (2006) Effects of dietary levels of protein on nitrogenous metabolism of Rhamdia quelen (Teleostei: Pimelodidae). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 145(2):181–187

Mohanta KN, Mohanty SN, Jena JK, Sahu NP (2008) Protein requirement of silver barb, Puntius gonionotus fingerlings. Aquaculture Nutrition 14(2):143–152. https://doi.org/10.1111/j.1365-2095.2007.00514.x

Mohapatra S, Chakraborty T, Prusty AK, Das P, Paniprasad K, Mohanta KN (2012) Use of different microbial probiotics in the diet of rohu, Labeo rohita fingerlings: effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora: Use of probiotics in the diet of rohu, Labeo rohita. Aquaculture Nutrition 18(1):1–11. https://doi.org/10.1111/j.1365-2095.2011.00866.x

Mrong CE, Islam MdR, Kole K, Neepa NN, Alam MdJ, Haque MdR, Rahman UO, Mostakim GM (2021) Malathion-Induced Hematoxicity and Its Recovery Pattern in Barbonymus gonionotus. Journal of Toxicology 2021:1–10. https://doi.org/10.1155/2021/9417380

Nandeesha MC, Srikanth GK, Keshavanath P, Varghese TJ, Basavaraja N, Das SK (1990) Effects of non-defatted silkworm-pupae in diets on the growth of common carp, Cyprinus carpio. Biological Wastes 33(1):17–23

Neepa NN, Mrong CE, Kole K, Sultana N, Islam MR, Mely SS, Mostakim GM (2022) Improvement of growth and hematological profile of juvenile silver barb (Barbonymus gonionotus) through dietary probiotic (Bacillus sp.) supplementation. Journal of Survey in Fisheries Sciences :11–25

Nelson DA, Morris MW, Todd JC, Sanford AH, Davidsohn I (1984) Hematology and coagulation. Clinical diagnosis and management by laboratory methods Japan: John Bernard Henry 1(984):589–92

Ochoa S (1955) Malic dehydrogenase and ‘malic’enzyme. Methods of enzymology 1:735–745

Olaniyi CO, Babasanmi GO (2013) Performance characteristics of African Cat fish (Clarias gariepinus) fed varying inclusion levels of silk worm pupae (Anaphe infracta). Bangladesh Journal of Animal Science 42(1):76–80

Oso JA, Iwalaye OA (2014) Growth performance and nutrient utilization efficiency of Clarias gariepinus juveniles fed Bombyx mori (mulberry silkworm) meal as a partial replacement for fishmeal. British Journal of Applied Science & Technology 4(26):3805–3812

Phillips AM (1972) Calorie and energy requirement. Fish nutrition :1–28

Pieper A, Pfeffer E (1980) Studies on the effect of increasing proportions of sucrose or gelatinized maize starch in diets for rainbow trout (Salmo gairdneri, R.) on the utilization of dietary energy and protein. Aquaculture 20(4):333–342

Raghuvaran N, Varghese T, Jana P, RJ AB, Sudarshan S, Alrashdi YBA, Ibrahim AE, El Deeb S (2024) Current Status and Global Research Trend Patterns of Insect Meal in Aquaculture From Scientometric Perspective:(2013–2022). Aquaculture Nutrition 2024:5466604

Rahman MA, Habib KA, Hossain MA, Azad SO, Rayhan MZ (2017) Impacts of stocking density and economic returns on the cage culture of stinging catfish, Heteropneustes fossilis. significance 1(T2):T3

Raja PK, Aanand S, Sampathkumar JS, Padmavathy P (2020) Effect of silkworm (Bombyx mori) pupae on the growth and maturation of rainbow shark Epalzeorhynchos frenatum (Fowler, 1934) under captive rearing. Indian J Fish 67(4):89–96

Rana KJ, Siriwardena S, Hasan MR (2009) Impact of rising feed ingredient prices on aquafeeds and aquaculture production

Rath RK (2018) Freshwater aquaculture. Scientific publishers

Rick W, Stegbauer HP (1974) α-Amylase measurement of reducing groups. In: Methods of enzymatic analysis. Elsevier, pp 885–890

Sa R, Pousao-Ferreira P, Oliva-Teles A (2006) Effect of dietary protein and lipid levels on growth and feed utilization of white sea bream (Diplodus sargus) juveniles. Aquaculture Nutrition 12(4):310–321. https://doi.org/10.1111/j.1365-2095.2006.00434.x

Salhi M, Bessonart M, Chediak G, Bellagamba M, Carnevia D (2004) Growth, feed utilization and body composition of black catfish, Rhamdia quelen, fry fed diets containing different protein and energy levels. Aquaculture 231(1–4):435–444

Santos WM, Costa LS, López-Olmeda JF, Costa NCS, Santos FAC, Oliveira CG, Guilherme HO, Bahiense RN, Luz RK, Ribeiro PAP (2020) Dietary protein modulates digestive enzyme activities and gene expression in red tilapia juveniles. Animal 14(9):1802–1810

Shakoori M, Gholipour H, Naseri S, Khara H (2016) Growth, survival, and body composition of rainbow trout, when dietary fish meal is replaced with silkworm () pupae. Fisheries & Aquatic Life 24(1):53–57

Shamna N, Sardar P, Sahu NP, Phulia V, Rajesh M, Fawole FJ, Pal AK, Angel G (2017) Heamato-immunological and physiological responses of Labeo rohita fingerlings to dietary fermented Jatropha curcas protein concentrate. Animal Feed Science and Technology 232:198–206

Shyama S (1990) Growth performance of carps in different diet treatment and under polyculture. PhD Thesis, Ph. D. Thesis. University of Agricultural Sciences, Bangalore

Siddiqui TQ, Khan MA (2009) Effects of dietary protein levels on growth, feed utilization, protein retention efficiency and body composition of young Heteropneustes fossilis (Bloch). Fish Physiol Biochem 35(3):479–488. https://doi.org/10.1007/s10695-008-9273-7

Silva RD da, Rocha LO, Fortes BDA, Vieira D, Fioravanti MCS (2012) Parâmetros hematológicos e bioquímicos da tilápia-do-Nilo (Oreochromis niloticus L.) sob estresse por exposição ao ar. Pesquisa Veterinária Brasileira 32:99–107

Singh SK, Pawar L, Thomas AJ, Debbarma R, Biswas P, Ningombam A, Devi AG, Waikhom G, Patel AB, Meena DK, Chakraborty G (2023) The current state of research and potential applications of insects for resource recovery and aquaculture feed. Environ Sci Pollut Res 31(53):62264–62282. https://doi.org/10.1007/s11356-023-29068-6

Spinelli J, Mahnken C, Steinberg M (1979) Alternate sources of proteins for fish meal in salmonid diets.

Stankus A (2021) State of world aquaculture 2020 and regional reviews: FAO webinar series. FAO aquaculture newsletter (63):17–18

Tibbetts SM, Lall SP, Milley JE (2005) Effects of dietary protein and lipid levels and DP DE-1 ratio on growth, feed utilization and hepatosomatic index of juvenile haddock, Melanogrammus aeglefinus L. Aquac Nutrition 11(1):67–75. https://doi.org/10.1111/j.1365-2095.2004.00326.x

Tok NC, Jain KK, Prabu DL, Sahu NP, Munilkumar S, Pal AK, Siddiah GM, Kumar P (2017) Metabolic and digestive enzyme activity of *Pangasianodon hypophthalmus* (Sauvage, 1878) fingerlings in response to

alternate feeding of different protein levels in the diet. Aquac Res 48(6):2895–2911. https://doi.org/10.1111/are.13122

Vijayan MM, Leatherland JF (1989) Cortisol-induced changes in plasma glucose, protein, and thyroid hormone levels, and liver glycogen content of coho salmon (*Oncorhynchus kisutch Walbaum*). Can J Zool 67(11):2746–2750. https://doi.org/10.1139/z89-389

Winfree RA, Stickney RR (1981) Effects of dietary protein and energy on growth, feed conversion efficiency and body composition of Tilapia aurea. The Journal of nutrition 111(6):1001–1012

Wooten IDP (1964) Microanalysis. Medical biochemistry 98:101–107

Wroblewski F, Ladue JS (1955) Serum glutamic-oxalacetic-transaminase activity as an index of liver-cell injury from cancer.A preliminary report. Cancer 8(6):1155–1163. https://doi.org/10.1002/1097-0142(1955)8:6<1155::AID-CNCR2820080611>3.0.CO;2-V

Wu X, Gatlin III DM (2014) Effects of altering dietary protein content in morning and evening feedings on growth and ammonia excretion of red drum (Sciaenops ocellatus). Aquaculture 434:33–37

Yamashiro D, Neu DH, Moro EB, Moro EB, Feiden A, Signor A, Boscolo WR, Bittencourt F (2016) Performance and muscular development of Nile tilapia larvae (Oreochromis niloticus) fed increasing concentrations of phenylalanine. Agricultural Sciences 7(12):900

Yang H, He J, Lv M, Huang G, Wen L, Bi X, Hu T, Ma H (2018) Preliminary effects of dietary protein levels on muscle quality and digestive enzyme activities in GIFT-Oreochromis niloticus. Aquaculture Studies 18(1):1–10

Ye C, Wu Y, Sun Z, Wang A (2017) Dietary protein requirement of juvenile obscure puffer, *T akifugu obscurus* . Aquac Res 48(5):2064–2073. https://doi.org/10.1111/are.13040

Yengkokpam S, Debnath D, Pal AK, Sahu NP, Jain KK, Norouzitallab P, Baruah K (2013) Short-term periodic feed deprivation in Labeo rohita fingerlings: effect on the activities of digestive, metabolic and anti-oxidative enzymes. Aquaculture 412:186–192

Zahan N, Hossain MA, Islam MR, Saha J, Akter T, Fatema UK, Haque F (2024) Effects of dietary Spirulina platensis on growth performance, body composition, haematology, immune response, and gut microflora of stinging catfish Heteropneustes fossilis. Aquaculture Reports 35:101997

Table 1. Ingredients (%) and proximate composition of experimental diets

Ingredients	**Control**	**T1-32.5%**	**T2-35%**	**T3-37.5%**	**T4-40%**	**T5-42.5%**	**T6-45%**
Fish meal	60	17	20.5	24	27.5	31	34.5
Silkworm pupae meal	0	17	20.5	24	27.5	31	34.5
Soybean meal	18	30	26	24	22	20	19
Rice bran	13	18	19	16	13	10	6
Cassava starch	5	16	12	10	8	6	4
Fish oil	3	1	1	1	1	1	1
Vitamin & mineral premix	1	1	1	1	1	1	1
Proximate composition							
Crude protein %	39.95	32.55	35.24	37.54	40.16	42.58	45.12
Crude lipid %	6.57	7.63	7.86	7.98	8.12	8.35	8.54
Fibre %	8.18	7.63	7.89	8.05	8.24	8.44	8.58
Moisture %	12.3	11.35	11.18	11.1	10.96	10.84	10.78
Ash %	12.3	8.26	8.21	8.16	8.12	8.09	8.06
Calorific value (MJ/Kg)	17.96	18.05	18.04	17.9	17.96	17.97	17.95

Table-3 shows Digestive enzyme activity

Table 2- Growth performance of *H. fossilis* fed with different experimental feed

Parameters	control	T1-32.5%	T2-35%	T3-37.5%	T4-40%	T5-42.5%	T6-45%
Mean initial weight (g)	0.652 ± 0.01	0.46 ± 0.01	0.463 ± 0.02	0.464 ± 0.01	0.462 ± 0.01	0.461 ± 0.01	0.467 ± 0.01
Mean final weight (g)	4.33 ± 0.16^{d}	0.37 ± 0.12^{e}	4.69 ± 0.18^{d}	5.68 ± 0.17^{c}	7.61 ± 0.14^{a}	6.90 ± 0.15^{b}	6.65 ± 0.12^{b}
Mean weight gain (g)	3.68 ± 0.17^{d}	2.90 ± 0.11^{e}	4.22 ± 0.20^{d}	5.23 ± 0.16^{c}	7.135 ± 0.12^{a}	6.445 ± 0.14^{b}	6.18 ± 0.011^{b}
Specific growth rate (%/day)	3.15 ± 0.36^{d}	3.30 ± 0.02^{d}	3.86 ± 0.14^{c}	4.17 ± 0.01^{b}	4.66 ± 0.02^{a}	4.51 ± 0.01^{a}	4.42 ± 0.07^{ab}
Food conversion ratio	1.72 ± 0.05^{a}	1.72 ± 0.05^{d}	1.62 ± 0.04^{bc}	1.56 ± 0.04^{b}	1.32 ± 0.03^{a}	1.40 ± 0.05^{a}	1.43 ± 0.04^{ab}
Protein efficiency ratio	1.46 ± 0.05^{b}	1.57 ± 0.01^{d}	1.67 ± 0.04^{c}	1.78 ± 0.03^{b}	1.94 ± 0.03^{a}	1.87 ± 0.01^{b}	1.83 ± 0.01^{b}
Survival	96.25 ± 1.76^{a}	97.5 ± 3.5^{a}	96.25 ± 1.76^{a}	99.00 ± 1.42^{a}	100.00 ± 0.00^{a}	98.75 ± 1.78^{a}	98.5 ± 1.4^{a}

Values are in mean ± S.D. Values in the same row with different superscript differ significantly ($p<0.05$) between the treatments.

Enzymes	T1-32.5%	T2-35%	T3-37.5%	T4-40%	T5-42.5%	T6-45%
Protease (Unit/mg protein/min)	0.95 ± 0.007^{e}	1.42 ± 0.02^{d}	1.57 ± 0.007^{d}	2.40 ± 0.04^{a}	2.03 ± 0.02^{b}	1.85 ± 0.04^{c}
Amylase (Unit/mg protein/min)	0.68 ± 0.03^{a}	0.62 ± 0.2^{b}	0.58 ± 0.01^{b}	0.50 ± 0.001^{c}	0.44 ± 0.01^{d}	0.41 ± 0.01^{d}
Lipase (Unit/mg protein/min)	1.35 ± 0.03^{f}	1.78 ± 0.01^{e}	1.92 ± 0.05^{d}	2.42 ± 0.3^{c}	2.57 ± 0.04^{b}	2.68 ± 0.20^{a}

Values are in mean ± S.D. Values in the same row with different superscripts differ significantly ($p<0.05$) between the groups.

Table-4 shows Metabolic enzyme activity

Enzymes	T1-32.5%	T2-35%	T3-37.5%	T4-40%	T5-42.5%	T6-45%
MDH (Unit/mg protein/min)	0.05 ± 0.001^{d}	0.08 ± 0.002^{d}	0.112 ± 0.005^{c}	0.138 ± 0.003^{a}	0.125 ± 0.006^{b}	0.121 ± 0.004^{b}
LDH (Unit/mg protein/min)	0.074 ± 0.002^{d}	0.095 ± 0.003^{d}	0.116 ± 0.001^{c}	0.142 ± 0.005^{a}	0.134 ± 0.002^{b}	0.128±0.002
AST (nm/mg/min)	12.48 ± 0.21	12.51 ±0.13	12.58 ± 0.11	12.71 ± 0.12	12.76 ± 0.04	12.79 ± 0.12
ALT (nm/mg/min)	15.35 ± 0.05	15.38 ±0.04	15.40 ± 0.02	15.41 ± 0.05	15.43 ± 0.06	15.46 ± 0.03

Values are in mean ± S.D. Values in the same row with different superscripts differ significantly ($p<0.05$) between the groups.

Table 5- Hematological parameters of the *H. fossilis* at the end of the experimental study

Parameters	control	T1-32.5%	T2-35%	T3-37.5%	T4-40%	T5-42.5%	T6-45%
RBC (cells/µl)	2.18±0.20	2.50±0.05[d]	2.4±0.04[c]	2.71±0.25[c]	3.58±0.05[a]	3.53±0.01[b]	3.50±0.02[b]
WBC (cells/µl)	26.1±1.52	25.9±1.52[d]	26.8±1.60[c]	27.2±0.34[c]	29.8±0.62[a]	28.2±0.4[b]	28.6±0.61[b]
HB (g/dl)	8.55±0.45	8.41±0.50[e]	8.75±0.25[d]	9.53±0.02[c]	10.16±0.13[a]	10.74±0.32[a]	10.71±0.30[b]
HCT (%)	15.5±0.25	15.68±0.01[e]	15.75±0.02[d]	16.35±0.03[d]	16.79±0.03[c]	17.89±0.02[ab]	17.77±0.01[b]
MCV (fl)	89.9±2.51	91.53±1.41[a]	90.7±0.45[b]	90.12±0.36[c]	88.61±0.11[d]	90.32±0.20[c]	90.26±0.20[c]
MCH (pg)	48.0±2.08	49.36±0.50	49.5±0.01	48.8±0.62	46.9±0.2	46.4±0.02	46.2±0.50
MCHC (g/dl)	58.9±0.03	58.26±0.37	57.6±0.66	60.9±0.32	68.04±1.3	66.15±0.52	65.24±0.16

Values are in mean ± S.D. Values in the same row with different superscripts differ significantly ($p<0.05$) between the groups.

Table 6- Whole body carcass composition of the *H.fossilis* at the end of the experimental study

Parameters	control	T1-32.5%	T2-35%	T3-37.5%	T4-40%	T5-42.5%	T6-45%
Crude protein %	15.75±0.03[d]	15.68±0.01[e]	15.75±0.02[d]	16.35±0.03[d]	16.79±0.03[c] [a]	17.89±0.02[ab]	17.77±0.01[b]
Crude lipid %	2.85±0.04[a]	2.96±0.01[d]	2.83±0.02[d]	2.76±0.02[c]	2.89±0.01[c]	3.32±0.02[b]	3.48±0.008[b]
Moisture %	74.33±0.15[a]	75.85±0.14	74.8±0.11[a]	73.81±0.03[b]	73.43±0.04[c]	72.86±0.03[d]	73.12±0.03[c]
Ash %	1.51±0.03	1.48±0.01	1.56±0.02	1.52±0.02	1.54±0.03	1.48±0.01	1.55±0.04

Values are in mean ± S.D. Values in the same row with different superscripts differ significantly ($p<0.05$) between the groups.

Table 7- Sensory attributes of the *H. fossilis* at the end of the experimental study

Parameters	T1-32.5%	T2-35%	T3-37.5%	T4-40%	T5-42.5%	T6-45%
Appearance	8.9 ± 0.2	9.0 ± 0.1	9.0 ± 0.2	9.0 ± 0.3	8.9 ± 0.1	9.0 ± 0.2
Odour	6.0 ± 0.31[a]	6.0 ± 0.1[a]	6.0 ± 0.3[a]	6.0 ± 0.1[a]	5.8 ± 0.2[a]	5.5 ± 0.1[b]
Flavour and taste	5.8 ± 0.3[a]	5.8 ± 0.2[a]	6.0 ± 0.3[a]	6.0 ± 0.3[a]	5.8 ± 0.4[a]	5.5 ± 0.3[b]
Juiciness	6.8 ± 0.2	6.8 ± 0.3	6.9 ± 0.0	7.0 ± 0.1	7.0 ± 0.1	7.0 ± 0.1
Overall acceptability	7.8 ± 1.5[a]	7.9 ± 0.8[a]	7.8 ± 0.9[a]	7.9 ± 1.2[a]	7.8 ± 0.8[a]	7.0 ± 1.2[b]

Values are in mean ± S.D. Values in the same row with different superscripts differ significantly ($p<0.05$) between the groups.

Table 8- Water quality parameters

SI. No	Parameters	Experimental study
		Mean ± SD
1.	**Temperature °C**	27.15 ± 0.5
2.	**P[H]**	7.15 ± 0.08
3.	**DO (ppm)**	5.45 ± 0.69

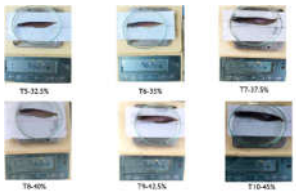
4.	**Alkalinity (ppm)**	58.3 ± 8.4
5.	**Hardness (ppm)**	97.0 ± 5.82
6.	**TDS (ppm)**	146 ± 0.05
7.	**Ammonia (ppm)**	0.06 ± 0.03
8.	**Nitrite (ppm)**	0.02 ± 0.07
9.	**Nitrate (ppm)**	3.71 ± 023
10.	**Inorganic phosphate**	0.02 ± 0.008

Values are in mean ± S. D

Drying of SWP meal

Different ingredients

Drying of feed



Fina recorded weight

Collection of intestines for enzyme analysis