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# PROTEIN REQUIREMENT OF *HORABAGRUS BRACHYSOMA* (GUNTHER 1864) AN ENDEMIC AND THREATENED CATFISH FROM WESTERN GHATS REGION

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**Abstract:** The effects of dietary protein levels on the growth and feed utilization of an endemic and threatened yellow catfish, *Horabagrus brachysoma* from peninsular India was investigated. Six dietary protein levels from 20 to 45% were tested for fingerling yellow catfish over a feeding period of 8 weeks. Weight gain, survival and feed efficiency were significantly influenced by dietary protein level. A linear increase in weight gain and feed efficiency was observed in response to dietary protein level from 20 to 35%, after which it declined with protein levels of 40 and 45%. Protein efficiency ratio also started to decrease when fish were fed diets with excess of 35% protein. Two slope broken-line analyses revealed that 37% protein is optimum for maximizing weight gain in *H. brachysoma* fingerlings when fed a practical diet.

***Key words:*** catfish, endemic, growth, *Horabagrus brachysoma*, protein.

## INTRODUCTION

*Horabagrus brachysoma* is an endemic yellow catfish having a restricted distribution in rivers, backwaters and associated wetlands of two South Indian states, Kerala and Karnataka (Ali *et al.,* 2007). Known locally as '*manjakoori*' the catfish is highly acclaimed as food fish due to its good taste and the young ones are widely accepted as an ornamental fish owing to its brilliant yellow colour. Multitude of stressors including over exploitation, habitat alteration and pollution has however, resulted in the population decline of *H. brachysoma* in its native ranges and the fish is listed as threatened (Molur *et al.,* 2011). In spite of its threatened status and declining numbers, commercial exploitation for both the food and ornamental markets still continue, and around 6.7 tons of the catfish was observed to be caught from Vembanad Lake during 2003-2004 (Sreeraj *et al.,* 2007). A recent study revealed that the population of *H. brachysoma* is over exploited in the river Periyar (Prasad *et al.,* 2012). Conservation and management of *H. brachysoma* is an immediate research priority in the region and aquaculture is widely opined as one of the most feasible options (Prasad, 2008).

The technology for captive breeding of *H. brachysoma* is currently available in some Asian countries including India but the availability of the stock material is considered to be a major constraint for its popularization (Giri *et al.* 2011). In nature, the yellow catfish is known to be omnivorous and euryphagous, feeding predominantly on crustaceans, small fishes and molluscs (Sreeraj *et al.,* 2006). The availability of food materials in their habitats is one of the critical factors affecting dietary intake and the species is known to be capable of widening its food spectrum if such a need arises (Sreeraj *et al*., 2006; Prasad and Ali, 2008). Preliminary investigation on the nutrition and feeding of *H. brachysoma* in captivity was revealed that the species responds well to both natural and formulated diets and could therefore be an ideal candidate for aquaculture (Hakkim and Prasad, 2006).

The adequate supply of nutrients is essential for proper development and growth, as well as the reproduction and maintenance of animal health. Therefore knowledge of nutritional requirement is essential for the formulation of the complete and balanced diets that enhance growth performance (Sampaio Zuonon *et al.,* 2013). Protein is a dietary macronutrient whose requirement is prioritized in nutritional studies; because it represents the highest cost component of a fish feed due to its impact on weight gain (Meyer and Fracallosi, 2004) as well as nitrogenous waste excretion (McGoogan and Gatlin, 2000). The dietary protein is also important for synthesis of enzymes, hormones and neurotransmitters, and thus it is essential for maintaining homeostatis and animal health ( NRC, 1993). Determining the optimum protein content of an aquaculture diet to the minimum needed to promote acceptable growth can provide economic and environmental benefits in the form of decreased production cost and improved water quality (Reigh *et al.,* 2002). There is no information till date on the requirements for any of the nutrients other than the one of protein for *H. brachysoma* reported by Giri *et al.* (2011). Such information is crucial for the future development of aquaculture of this endemic catfish. The objective of the present study was therefore to determine the optimum dietary protein level for growth of young *H. brachysoma*.

## MATERIALS AND METHODS

Eighteen 30L cylindrical fibre reinforced plastic (FRP) tubs (35 x 25cm) connected to a temperature controlled; (28 ± 1ºC) water recycling system was used for the experiment. Water was supplied to each tub at a rate of 1L min$^{-1}$ from a 400 L overhead storage tank after dechlorination. A photoperiod of 12h L: 12h D was maintained throughout the experiment, with the period from 08:00 to 20:00 maintained as the light hours. Water quality parameters including pH (6.5 to 7.4) and dissolved oxygen (6.4 to 7.6 mg L$^{-1}$) were measured daily and ammonia (0.09 to 0.35 mg L$^{-1}$), nitrate (0.42 to 5.84 mg L$^{-1}$) and nitrite (0.02 to 0.26 mg L$^{-1}$) were measured weekly. The water quality parameters were within the acceptable range reported for this fish in captivity (Giri *et al.* 2011).

Early fingerlings of *H.brachysoma*, obtained from a hatchery in Alappuzha district, Kerala, India were transported to the laboratory at the Department of Zoology, University of Kerala, where the rearing experiment was subsequently carried out. They were acclimated to the experimental conditions and fed a mixture of fresh fish and shrimp for three days and subsequently weaned to a formulated diet (experimental control diet), for a week till the commencement of the feeding experiment. At the beginning of the experiment, all fish were pooled after one day of food deprivation. Fish were batch weighed and distributed into the eighteen rearing tanks (15 fish with a mean individual weight of 3.50 ± 0.82g in each tank).

All ingredients (fishmeal, soybean meal, wheat flour, fish oil and tapioca powder) were purchased from local markets and their proximate composition is determined by standard methods (AOAC 1999). Vitamin and mineral mixtures were formulated based on an earlier study on bagrid catfish (Ng et al. 2001). Six diets were formulated to contain 20, 25, 30, 35, 40 and 45% protein and 8% lipid by dry weight. Locally available fishmeal was used as the major protein source, fish oil as the lipid source and wheat flour as the carbohydrate source (Table 1). All ingredients were thoroughly mixed and made into pellets of two sizes (1 and 2 mm in diameter) with a laboratory pellet presser. All diets were oven-dried at 50º C and stored at 4ºC until further use.

Triplicate tanks were randomly assigned to each of the six dietary treatments (A to F). Fish were hand-fed to apparent satiation twice daily at 08:00 and 15:00h. Pellets were slowly fed to the fish to minimize feed wastage. Uneaten feed was siphoned out of each tank 30 minutes after each feeding, and the quantity estimated after oven drying them for 24h at 60ºC. Fish was also examined twice a day for general activity, physical and behavioral abnormalities. Ten fish from the initial population were collected and frozen at -

20°C for analysis of body composition. At the beginning (day 1) and end (day 56) of the trial, fish in each tank were counted and batch-weighed. Daily intake dry matter (DM) was determined by subtracting the residue from the feed supplied. Organic matter was calculated by subtracting total ash value from dry matter. Crude protein content was determined by Kjeldahl method and crude lipid content by ether-extraction method. Moisture content was estimated by drying at 105°C for 24 h in a hot air oven and caloric content by a bomb calorimeter and ash content by burning samples in a muffle furnace (AOAC 1999). The nitrogen free extract (NFE) of feeds were taken as the total carbohydrates assimilated by the fish and which is considered by subtracting protein, lipid and fibre from the organic matter. Growth performance and feed utilization efficiency were determined after recording final body weight. The following formulae were used for the determination of growth parameters and feed utilization (Giri *et al.* 2011):

Net weight gain (g) = $(W_f) – W_i$

Specific growth rate = $100 (Ln (W_f) ) Ln (W_i)) T)^{-1}$

Feed conversion ratio = $W_{TFC} (W_f) W_i))^{-1}$

Protein efficiency ratio = $(W_f) W_i) W)^{-1}_{prot,f}$

where $W_i$ and $W_f$ are the initial and final body weight (g), $_{WTFC}$ is the weight of feed consumed (g), T is duration of the experiment (days) and $W_{prot,f}$ is the weight of intake of dietary crude protein. Data were analyzed using one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ($P<$ 0.05) using the computer program STATISTICA 6.0. Two-slope broken-line analysis (Robbins *et al.* 1979) was used to determine optimal requirement of protein.

## RESULTS AND DISCUSSION

The table 2 shows the growth performance, survival and feed utilization of *H. brachysoma* fed diets containing different protein levels for a period of 8 weeks. Survival was not significantly affected by dietary protein levels (Table 2). Within the test range, weight gain increased significantly *($P < 0.05$)* up to a dietary protein level of 35%; beyond which it decreased significantly *($P$*<0.05). Broken line analysis (Figure 1) indicated that the dietary protein requirement for *H. brachysoma* based on percentage weight gain was 37%. Specific growth rate (SGR) was also highest among fish fed 35% protein diet and decreased thereafter. Feed efficiency (FE) ranged from 0.38 to 0.52, increased as dietary protein increased up to 35% and then decreased at 40 and 45% protein levels. However, FE in groups fed diets 40 and 45% CP was significantly higher *($p$*<0.05) compared to those fed low protein diet (20%). Protein efficiency ratio (PER) showed no clear trend in relation to dietary protein levels although they were significantly different between treatments (Table 2).

The results of the present study demonstrated that the level of dietary protein influenced growth performance and feed utilization in *H. brachysoma*. It was evident that *H. brachysoma* did not have enough protein for growth when fed with 20, 25 and 30% protein diets. These low protein diets resulted in lower weight gain when compared to the 35% diet. A dose-response relationship, where growth increased in relation to increases in dietary protein content up to 35% after which it decreased with further increase in protein level was observed in the present study.

Increases in dietary protein have often been associated with higher weight gains and growth rates in many species, especially in carnivorous fish (NRC, 1993). However, there exist protein levels, beyond which growth ceases, and sometimes decrease (Chong *et al.,* 2000; Yang *et al.* 2002). Such a growth depression in response to protein levels higher than the optimum was observed in the present study which could be due to the energy costs of deamination of excess amino acids (Jauncey, 1982). Inclusion of dietary protein levels beyond the optimum level also results in high level of ammonia production affecting the voluntary feed intake as well as growth (McGoogan and Gatlin, 2000). In the present study, highest SGR was also obtained among the fish fed 35% protein diet and

**Table 1.** Formulation and chemical composition[1] of the experimental diets(g $kg^{-1}$ dry matter)

| Ingredients | Diets | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | F |
| Fishmeal (Indian) | 140 | 210 | 270 | 340 | 410 | 460 |
| Soybean meal[2] | 60 | 90 | 150 | 200 | 250 | 310 |
| Wheat flour | 650 | 550 | 430 | 320 | 200 | 90 |
| Tapioca powder | 30 | 30 | 30 | 30 | 30 | 30 |
| Fish oil | 60 | 60 | 60 | 50 | 50 | 50 |
| Mineral mix[3] | 30 | 30 | 30 | 30 | 30 | 30 |
| Vitamin mix[4] | 20 | 20 | 20 | 20 | 20 | 20 |
| Chromic oxide | 10 | 10 | 10 | 10 | 10 | 10 |
| ***Analysed chemical composition*** **(g $kg^{-1}$ dry diet)** | | | | | | |
| Crude Protein | 210 | 255 | 304 | 356 | 408 | 452 |
| Crude Lipid | 83 | 86 | 88 | 81 | 83 | 84 |
| Dry Matter Ash | 31 | 32 | 32 | 34 | 36 | 37 |
| Gross energy ($kJg^{-1}$) | 20.1 | 20.7 | 20.7 | 20.9 | 21.1 | 21.0 |
| PE ratio[5] | 10.44 | 12.31 | 14.68 | 17.03 | 19.33 | 21.52 |

[1]means of triplicates

[2]solvent extracted and de hulled

[3]Mineral mix (g $kg)^{-1}$ diet: calcium phosphate 270.98; calcium lactate 327; ferrous sulphate 25; magnesium sulphate 132; potassium chloride 50; sodium chloride 60; potassium iodide 0.15; copper sulphate 0.785; manganese oxide 0.8; cobalt carbonate 1; zinc oxide 3; sodium selenite 0.011; calcium carbonate 129.27

[4]Vitamin mix (mg $kg)^{-1}$ diet: ascorbic acid 45; inositol 5; choline chloride 75; niacin 4.5; riboflavin 1; pyridoxine.HCl 1; thiamine mononitrate 0.92; calcium D-pantothenate 3; retinyl acetate 0.6; cholecalciferol 0.083; menadione sodium bisulphite 1.67; DL-a-tocopheryl acetate (powder 500 IU g)1) 8; D-biotin 0.02; folic acid 0.09; vitamin B12 0.00135; cellulose 854.11.

[5]Protein: energy ratio - mg protein $kJ^{-1}$ gross energy

decreased thereafter. A similar observation was reported by Giri *et al.* (2011) for this fish, where the maximum growth rate was reported at 35% protein in the diet although the practical optimum protein requirement was 39%. SGR, apart from being an indicator of nutritional state, is also a good indicator of protein quality, and under controlled conditions the weight gain is proportional to essential amino acids provided (Tacon 1989).

As reported by Ng *et al*. (2001) in fingerlings of *Mystus nemurus*, FE values among *H. brachysoma* fingerlings in the present study also showed a linear increase when fed dietary protein level of 20% to the optimum level of 35% and thereafter declined. Such decreases in FE when fed protein levels beyond optimum have been seen in many species of fish and has been attributed to the diversion of dietary energy available for growth to deaminate and excrete excess absorbed amino acids (Jauncey, 1982).

Protein efficiency ratio is influenced by dietary protein level and its effects vary with species (Dabrowski 1979). Although PER of fish fed different dietary levels of protein were significantly different in the present study, a clear trend was generally absent. However, PER was seen to decrease in relation to protein levels above

**Table 2.** Growth performance and feed utilization of *H. brachysoma* fed various levels of dietary protein for 56 days[1]

| DP[2] | PWG[3] | SUR[4] | SGR[5] | FE[6] | PER[7] |
|---|---|---|---|---|---|
| 20 | 161[a] | 82[a] | 1.727[a] | 0.38[a] | 1.44[d] |
| 25 | 180[b] | 82[a] | 1.854[b] | 0.44[b] | 1.41[c] |
| 30 | 202[de] | 84[a] | 1.974[de] | 0.48[d] | 1.40[b] |
| 35 | 209[e] | 86[a] | 2.014[e] | 0.52[e] | 1.42[cd] |
| 40 | 195[cd] | 82[a] | 1.939[cd] | 0.46[c] | 1.40[b] |
| 45 | 188[bc] | 82[a] | 1.900[bc] | 0.44[b] | 1.29[a] |

[1]Values are the mean of triplicate groups of five fishes. Mean values in columns with different superscripts are significantly different (P <0.05)

[2] Dietary Protein (% dry matter)

[3] Percentage weight Gain (%) = Expressed as the percentage of initial body weight at the end of 56 days.

[4]Survival (%) = 100 (Number of dead fish / number of initial fish)

[5]Specific Growth Rate (% /day) = (In Final Weight – In Initial Weight) x 100 / t

[6]Feed Efficiency = fresh body weight gain (g) / dry feed intake (g)

[7]Protein Efficiency Ratio = fresh body weight gain (g) / total protein intake (g)

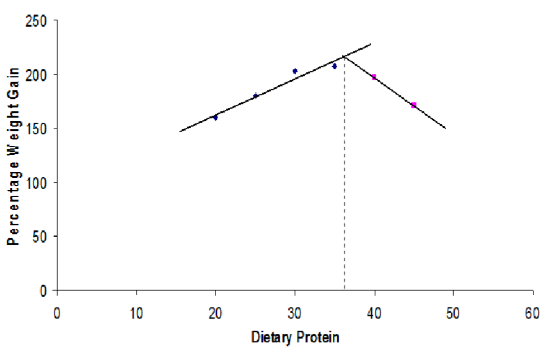


**Fig. 1.** Broken-line analysis showing estimation of the optimum dietary protein requirement for *H. brachysoma* fingerlings based on percentage weight gain.

the optimum as was observed by earlier workers (Chong *et al.,* 2000; Ng *et al.* 2001).

The optimal protein requirement of *H. brachysoma* which was determined as 37% is similar to that of the silver catfish, *Rhamdia quelen* 37% (Salhi *et al.* 2004), green catfish, *Mystus nemurus* 35% (Afini and Hashim 2004) and dwarf catfish *Clarias isheriensis* 37% (Fagbenro 1992). The optimal protein requirement for *Mystus nemurus* is however known to fall under a broad range of 35% to 44% (Ng *et al.,* 2001, Khan*et al.*1993 and Afini, and Hashim 2004), apparent differences attributed to the different fish sizes used, quality of the protein tested and the variations in dietary protein to lipid ratio.

The protein requirement of fingerling *H. brachysoma* is however on a higher side when compared to other omnivorous catfish species including channel catfish, *Ictalurus punctatus* 28–32% (Robinson and Li, 2002) and stinging catfish, *Heteropneustes fossilis* 28-35% (Akand *et al.,* 1989), but lower than carnivorous catfishes including Chinese yellow catfish, *Pelteobagrus fulvidraco* 42% (Kim and Lee, 2005), African Vundu catfish, *Heterobranchus longifilis* 42.5% (Fagbenro *et al.* 1992) and African sharptooth catfish, *Clarias gariepinus* 43% (Ali and Jauncey, 2005).

The dietary protein requirement of *H. brachysoma* as determined in the present experiment is in accordance with its feeding habit in the wild fish, that of an omnivore (Sreeraj *et al.*, 2006; Prasad and Ali, 2008). Ng *et al.* (2001) opined that the protein requirement of fish can sometimes be overestimated when practical diets are used in the formulation because factors such as the digestibility and amino acid composition of the dietary proteins are not always given adequate consideration. Taking this factor into consideration, the protein requirement of *H. brachysoma* could be lower than the level found as optimal in the present study when purified ingredients are used. Future studies should therefore investigate the effects of protein quality (digestibility and amino acid requirements) and protein to energy and lipid ratios to quantify the exact protein requirement of this species and arrive at a clear inference. The present study provides information on the protein requirements of the yellow catfish, *H. brachysoma* at young stages and suggests 37% as the optimum protein levels and 35% minimum to be incorporated into practical diets for commercial rearing purpose to achieve fast growth and maximum survival.

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