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Note

Effect of dietary protein levels on the growth of Carnatic carp *Barbodes carnaticus* (Jerdon, 1849) fingerlings

N. SRIDHAR, B. GANGADHAR, UMALATHA, C. H. RAGHAVENDRA, S. S. GIRI* AND P. JAYASANKAR*
Regional Research Centre of ICAR-Central Institute of Freshwater Aquaculture, Hesaraghatta Lake P.O. Bangalore - 560 089, Karnataka, India
**ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar - 751 002, Odisha, India*
e-mail: sridharcifa@rediffmail.com

ABSTRACT

Fingerlings of *Barbodes carnaticus* (Jerdon 1849) (6.40±0.267 cm, 2.46±0.39 g) were reared in aerated plastic tubs (40 l) with five iso-energetic diets formulated to contain crude protein levels ranging from 25 to 45% using pure ingredients. Initially, the diet was fed at @5% of the biomass and gradually adjusted based on daily feed consumption. The fish were allowed to feed for 6 h and thereafter the unconsumed feeds were siphoned out. The following day, faecal matter was collected from each tank by filtering the water with a fine meshed nylon cloth (15 µm), dried, pooled and stored for proximate analysis. Water from each tub was replaced with 50% freshwater every day after faecal matter collection. The feeding trial was conducted for a period of 60 days. Proximate composition of feed and faecal matter was analysed. Acid insoluble ash was used as the reference marker for dry matter, protein and fat digestibility determination. The growth parameters and protein efficiency ratio (PER) were higher ($p<0.05$) in fish fed 35% protein diet compared to other diets. The feed conversion ratio (FCR) was lower ($p<0.05$) in 35, 40 and 45% protein diets compared to the lower protein diets. The carcass proximate composition revealed the lowest moisture ($p<0.05$) and highest protein ($p>0.05$) and fat content ($p<0.05$) in fish fed 35% protein diet compared to other diets. The digestibility of dry matter and nutrients was higher ($p<0.05$) with 35% protein diet. The study revealed crude protein requirement of 35% by the fingerlings of *B. carnaticus*.

Keywords: *Barbodes carnaticus,* Dietary protein, Fingerlings

Indian major carps contribute the lion's share to freshwater aquaculture production in India, around 80% by volume. India is regarded as a 'carp country' due to the rich diversity of carps in its freshwater ecosystems. The country is blessed with 15-20 varieties of minor carps that have a huge potential for freshwater aquaculture, yet to be exploited. A need for diversification of farmed fish species has been emphasised (NACA/FAO, 2000). These carp species, some of which belong to the minor carps, can be considered as alternatives to the major carp species for diversification in freshwater aquaculture. *Barbodes carnaticus* (Jerdon 1849), commonly known as Carnatic carp, is one such species endemic to the Western Ghats of India. The breeding technology of the species has already been standardised at the Regional Research Centre of ICAR-Central Institute of Freshwater Aquaculture, Bangalore. *B. carnaticus* is characterised by an elongated and stocky body. Compared to other members of the genus, this species attains larger size; the maximum weight recorded being 12 kg and length 90 cm (Talwar and Jhingran, 1991). The higher growth rate of *B. carnaticus* in the first year of its life span along with other favourable characteristics makes this species an excellent candidate for freshwater aquaculture (Manojkumar and Kurup, 2010).

Proteins are the structural components and energy source in diets of fish and play a key role as they are very essential for body maintenance and growth. Generally, fish requires higher levels of protein in diets and this nutrient is the costliest dietary constituent. A significant reduction in feed cost can be achieved if diets with low protein could be fed to fish without compromising growth and health (Webster *et al.,* 2000). However, inadequate protein in the diet results in reduction of growth. On the other hand, if too much protein is supplied in the diet, only part of it will be used to make new proteins and the remainder will be converted to energy (Wilson, 2002). Hence determination of optimum protein requirement for fish is the first step to the development of low-cost feeds. Earlier studies have given an estimate of the protein requirement of Indian cultivable carps using purified diets (Sen *et al.,* 1978; Singh and Sinha, 1981; Rao, 1987; Singh *et al.,* 1987; Singh and Bhanot, 1988; Swamy and Mohanty, 1988; Swamy *et al.,* 1988; Mohanty *et al.,* 1990). Jena *et al.* (2012) reported the protein requirement of the minor carp *Labeo fimbriatus*, to be 30.14% based on live weight gain. This study analysed the protein requirement of *B. carnaticus* as information pertaining to the protein requirement of a species is essential before it is introduced into the culture system.

For the present experiment, healthy fingerlings of *B. carnaticus* (n=180) having an average initial length of 6.40±0.267 cm and weight of 2.46 ± 0.39 g were distributed into groups of 12 fish each and stocked randomly into 15 aerated plastic tubs (40 l). The fish were maintained under control feed for a week. Five feeds were formulated to contain crude protein (CP) levels ranging from 25 to 45% using pure ingredients such as casein, gelatin, dextrin, carboxy methyl cellulose, cellulose and agar (Table 1). The ingredients were thoroughly mixed in the desired proportions in a mechanical mixer with hot water and cod liver oil and vitamin mixtures added after cooling. The dough was pelletised using a hand pelletiser fitted with a 1 mm die and the extruded noodles were sun dried (to <10% moisture). The sun dried feed were manually broken into small pieces and stored at room temperature (26±2ºC) in separate airtight containers till further use.

The experimental feeds prepared were given at 5% of the biomass initially and gradually adjusted based on the observation of daily feed consumption. The fish were allowed to feed for 6 h. The unconsumed feeds were siphoned out at the end of the feeding period. On the following day's faecal matter was collected from each tank by filtering the water with a fine meshed nylon cloth (15 µm), dried, pooled and stored for proximate analysis. About 50% of water from each aquarium was replaced with freshwater every day after faecal matter collection. This feeding and faecal matter collection trial was conducted for a period of 60 days. Proximate composition of feed and faecal matter was analysed (AOAC, 1995). Nitrogen free extract was calculated based on the 'difference method' (Hastings, 1976). Acid insoluble ash (AIA) was used as the reference marker (Goddard and McLean, 2001; Li *et al.,* 2008; Bob-Manuel, 2013) for dry matter, protein and fat digestibility determination (Maynard and Loosli, 1972).

$$\text{Total dry matter digestibility (\%)} = 100 - \left[100 \times \frac{\text{\% AIA in feed}}{\text{\% AIA in faeces}}\right]$$

$$\text{Nutrient digestibility (\%)} = 100 - 100 \left[\frac{\text{\% AIA in feed}}{\text{\% AIA in faeces}}\right] \times \left[\frac{\text{\% Nutrient in faeces}}{\text{\% Nutrient in feed}}\right]$$

Table 1. Ingredient proportion (%) and proximate composition (%, mean±SD) of experimental feeds.

Crude protein (%)	CP 25%	CP 30%	CP 35%	CP 40%	CP 45%
Ingredients					
Casein	25	28.59	33.36	38.13	42.89
Gelatin	5	5.91	6.64	7.59	8.54
Dextrin	30.1	30.61	23.36	15.9	8.46
Cod liver oil	8	8	8	8	8
CM Cellulose	12	12	12	12	12
Agar	2.5	2.5	2.5	2.5	2.5
Vitamin mineral mix	4.5	4.5	4.5	4.5	4.5
Cellulose	12.9	7.89	9.64	11.38	13.11
Proximate composition (%)					
Moisture	1.78±0.31	1.29±0.07	1.51±0.04	1.48±0.06	1.89±0.00
Crude protein	25.01±0.13	30.30±0.30	34.77±0.84	39.00±0.37	44.51±0.74
Fat	7.66±0.40	7.91±0.56	6.55±0.18	6.04±0.65	6.77±0.06
Ash	8.41±0.22	8.49±0.19	8.45±0.14	8.65±0.08	8.71±0.02
Crude fibre	7.48±0.00	6.23±0.26	5.23±0.16	6.71±0.11	7.68±0.38
NFE	49.66±0.28	45.78±0.74	43.49±1.26	38.12±1.14	30.44±1.18
Gross energy (kJg^{-1})	17.17	17.79	18.28	17.72	17.93

CP - Crude protein, NFE - Nitrogen free extract

The energy value of feed was obtained by multiplying protein, lipid and carbohydrate contents by factors 22.6, 38.9 and 17.2 respectively (Mayes, 1990) and expressed in kJ g^{-1}. During the experiment, water quality parameters, namely pH, temperature, dissolved oxygen and total alkalinity were analysed at fortnightly intervals following standard methods (APHA, 1998). Water samples were collected between 09.00 and 10.00 hrs.

At the end of the experiment, the length and weight of the fishes were measured. The weight gained by the fingerlings, specific growth rate (SGR) and feed conversion ratio (FCR) for each feed were calculated. Data was analysed using one-way ANOVA. Whenever significant difference (p<0.05) was noticed, Duncan's multiple range test was done (Duncan, 1955) and results presented as mean ± standard deviation.

The water quality parameters recorded during the experiment are given in Table 2. The growth parameters and protein efficiency ratio were higher ($p<0.05$) in fish fed 35% protein diet compared to the other diets (Table 3). The FCR was lower ($p<0.05$) in 35, 40 and 45% protein diets compared to the lower protein diets. No mortality of fish was observed in any of the experimental tubs during the study.

The carcass proximate composition revealed lowest moisture ($p<0.05$) and highest protein content ($p>0.05$) in fish fed 35% protein diet, there being no difference ($p>0.05$) among the other treatments (Table 4). Further, the fat content ($p<0.05$) was also higher in fish fed 35% protein diet compared to other diets. The ash content was higher ($p<0.05$) with 25 and 30% protein diets.

The digestibility of dry matter (DMD) and nutrients showed higher ($p<0.05$) values with 35% dietary protein (Fig. 1). While the total DMD showed a hyperbolic trend, the protein and nitrogen-free extract (NFE) digestibility showed an increasing and the fat digestibility a decreasing trend with the dietary protein level.

Studies with respect to protein requirements in fish have been conducted in many species before they were introduced in to the aquaculture system. Delong *et al.* (1958) were the first to use casein-gelatin based purified diets for studying the protein requirement of fish. Garling and Wilson (1976) reported a level of 25-36% crude protein in diets as optimum for warm water fishes. Fingerlings of Indian major carps

Table 4. Carcass composition (% on wet weight basis, mean±SD) of *B. carnaticus*

Feeds	Moisture	Crude protein	Fat	Ash
CP 25%	75.97±0.57[b]	16.76±0.40[a]	3.14±0.18[a]	3.62±0.08[c]
CP 30%	76.05±0.32[b]	16.88±0.20[ab]	3.24±0.12[ab]	3.59±0.05[cb]
CP 35%	74.43±0.24[a]	17.55±0.19[b]	4.38±0.00[e]	3.41±0.03[a]
CP 40%	75.94±0.36[b]	17.00±0.14[ab]	3.58±0.06[bd]	3.32±0.04[a]
CP 45%	75.74±0.42[b]	17.01±0.30[ab]	3.39±0.20[ab]	3.43±0.08[ab]

Table 2. Water quality parameters (mean±SD) recorded in the experimental tubs

Parameter	CP 25%	CP 30%	CP 35%	CP 40%	CP 45%
Temperature (°C)	25.1±0.319	24.9±0.45	25.6±0.23	24.7±0.01	25.3±0.14
pH (range)	7.28-7.98	7.29-8.00	7.41-8.10	7.35-8.00	7.31-8.05
Dissolved oxygen (ppm)	5.86±1.11	6.87±1.09	7.16±1.12	7.86±0.78	5.67±0.49
Total alkalinity (ppm)	323.01±8.31	327.87±7.61	338.78±6.81	330.16±5.26	331.49±3.18

Table 3. Growth parameters (mean ± SD) of *B. carnaticus* recorded during the study

Growth parameters	CP 25%	CP 30%	CP 35%	CP 40%	CP 45%
Initial weight (mg)	2.38±0.05	2.42±0.05	2.34±0.01	2.45±0.12	2.47±0.11
Final weight (mg)	3.22±0.18[a]	3.23±0.07[a]	3.79±0.21[b]	3.73±0.13[b]	3.57±0.12[ab]
Weight gain (%)	35.28±9.86[a]	33.27± 4.43[a]	61.95±9.18[b]	52.51±4.8[ab]	45.02±7.10[ab]
SGR (%)	0.50±0.12[a]	0.48±0.05[a]	0.80±0.09[b]	0.70±0.05[ab]	0.62±0.08[ab]
Feed conversion ratio	6.00±1.42[b]	6.00±0.61[b]	3.00±0.24[a]	3.34±0.37[a]	3.73±0.35[a]
Protein efficiency ratio	0.69±0.18[ab]	0.55±0.06[a]	0.96±0.08[bc]	0.77±0.09[ab]	0.60±0.06[a]

Figures in the same row with different superscripts are significantly different ($p<0.05$).

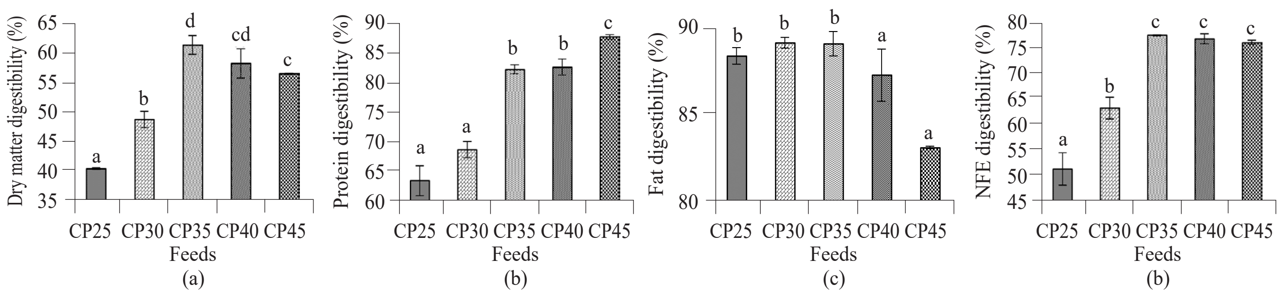

Fig. 1. Digestibility (mean±SD) of feed (DM) and nutrients (a: Dry matter; b: Protein; c: Fat; d: NFE) in *B. carnaticus* fed experimental feeds. Different alphabets on bars indicate statistical difference ($p<0.05$)

require around 30% dietary protein for growth (Renukaradhya and Varghese, 1986; Murthy, 2003; Singh *et al.,* 2006). Varghese *et al.* (1976) and Dabrowski (1977) reported the protein requirement of common carp and grass carp to be 31% and 41-43% respectively. *L. fimbriatus*, a medium carp has a dietary protein requirement of 30% based on live weight gain (Jena *et al.,* 2012). In the present study, the growth parameters and protein efficiency ratio were higher ($p<0.05$) in fish fed 35% protein diet. Variation in dietary protein requirement of fish is due to difference in species, feeding habit, size (age) of the fish, water temperature, feed quality, feed composition, biological value of protein, source of non-protein energy and availability of natural feed (Halver *et al.,* 1964; Garling and Wilson, 1976; Mazid *et al.,* 1979; Dabrowski *et al.,* 1989).

The growth parameters of *B. carnaticus*, feed conversion ratio and protein efficiency ratio showed a negative trend ($p>0.05$) above 35% protein level indicating more than the optimum level of dietary protein having adverse effects on growth of the species. Similar observation has been reported for catla (Dars *et al.,* 2010), rohu (Nandeesha *et al.,* 1991; Khan and Jafri, 1992; Chakraborthy *et al.,* 1999), grass carp (Dabrowsky*et al.,*1977) and stunted fingerlings of rohu (Kumar *et al.,* 2011) and catla (Ramaswamy *et al.,* 2013). This decreased growth may be attributed to the metabolic stress mediated by excess nitrogen content.

PER is known to decrease with increasing dietary protein content, indicating decrease in protein utilisation with increasing dietary protein levels (Jauncey, 1982; De Silva *et al.,* 1989; Khattab *et al.,* 2000; Kumar *et al.,* 2011). This is mainly because more dietary protein is used as energy when high protein diets are fed to fish (Kim *et al.,*1991). However, in the present study, decrease in PER was observed only above 35% dietary protein level. Dabrowski (1979) opined that the relationship between dietary protein and PER differs from species to species.

The carcass composition of *B. carnaticus* was influenced significantly by dietary protein level. Fish fed 35% protein diet had lower content of moisture and higher protein and lipid than fish fed other diets. Singh *et al.* (2006) also reported highest amount of protein and lipid in the muscle of rohu at optimum dietary protein level. Carcass composition is known to be influenced by many factors like geographical location, age, sex, maturity and feeding conditions. An increase in muscle protein and a decrease in lipid content with increasing dietary protein was noted by Dabrowski (1977) in grass carp, Jauncey (1982) in *Sarotherodon (=Oreochromis) mossambicus*, Fah and Leng (1986) in guppy, *Poecilia reticulata*, Shiau and Huang (1989) in hybrid tilapia (*Oreochromis niloticus* x *O. aureus*), Kheir (1997) and Al-Hafedh (1999) in Nile tilapia (*O. aureus*). However, such a trend was not observed in the present study. The carcass ash content was higher in fish fed 25 and 30% protein diets compared to other treatments. Khattab *et al.* (2000) reported that ash content was unaffected by protein level in Nile tilapia.

The total DMD was higher with high protein diets. Ginindza (2012) recorded a positive correlation between apparent digestibility coefficients of protein and lipid with dietary protein level. High protein and lipid digestibility with increase in dietary protein content was also observed in *Labeo rohita* (Gul *et al.,* 2007). In another study with rohu, Singh *et al*. (2005) reported that digestibility of protein was significantly higher in diets containing 30 and 35% crude protein, however, with further increase or decrease in protein level, a significant decrease in apparent protein digestibility was observed. In a study with grass carp, the increase in dietary protein, did not affect protein, lipid and NFE digestibility (Koprucu, 2012). In the present study, the protein digestibility was the highest with high protein diets. However, lipid digestibility coefficients were the highest with lowest protein diet (25%). The NFE digestibility was higher with high protein diets, this is particularly interesting due to the fact that these diets had low dietary NFE. It may be noted that the digestibility coefficients of DM and all the nutrients were the best with dietary crude protein level of 35%, corresponding with the fish growth.

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