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Dietary Protein Inclusion to Assess Growth and Feed Utilization in *Tor tor* (Hamilton, 1822)

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Abstract

The present research work embodies the nutritional requirement studies on *Tor tor* collected from the Mahanadi waters in Madhya Pradesh (India). During the present experiment, *Tor tor* fry with an average length and weight of 44.5 mm and 2.102 g respectively were subjected to 3 experimental feeds containing 25 % CP (T-1), 30 % CP (T-2) and 40 % CP (T-3), with a control group fed on feed containing 20 % CP. The results revealed the highest increment in overall length and weight in the case of T-3 (40 % CP), with as high as 98.5±3.5 mm and 6.317±1.1 gms, over a period of 12 months (365 days). Similarly, the highest values for SGR (0.145±0.002) and PER (0.846±0.02) were recorded in the case of T-3. Finally, the best feed composition of T-3 was documented by the lowest FCR value of 0.82±0.02.

Keywords: *Tor tor*, crude protein, SGR, FCR, PER

Introduction

Tor tor is the most common Himalayan mahseer and a very attractive sport fish with excellent food value. It is also a highly nutritious fish with good economic value. It has shown a steady decline in abundance in reservoirs in India. It inhabits riverine pools and lakes, and also streams with good flows and a rocky bottom, where they attain the best growth. They are benthopelagic, potamodromous and occur in tropical freshwaters (15 - 30 °C) at depths of up to 15 m. Adults have omnivorous feeding habits and feed on small fish, insects, molluscs, zooplankton, debris, sand, mud, fish scales and bones, fruits, chironomid larvae, water beetles, crustaceans, filamentous algae and macrophytes. Juveniles mainly consume insects [1]. Among Indian mahseers, *Tor tor* is the most important food and game fish in India after *Tor putitora* [2].

Studies on the nutritional aspects from a culture viewpoint related to conservation and propagation, though important, are very limited, particularly for Narmada mahseer. Attempts have been made to raise fry of putitora mahseer on formulated diets at the National Research Centre on Cold Water Fisheries (NRCCWF), while in case of khudree mahseer (*Tor khudree*) more systematic studies have been conducted to evaluate the optimum protein requirement [3], protein sources [4] and protein sparing effects of sardine oil [5]. During different stages of mahseer development, the protein requirement by this species needs to be understood for modifying/formulating the feed in order to make it more balanced and nutritive.

The present study has aimed to generate baseline data on nutrient requirements so as to develop appropriate feeds to enhance growth of mahseer in captivity under aquaculture conditions in order to increase production. At present there is only limited knowledge on the nutritional requirement of mahseer *Tor tor* when cultured in captivity in tropical waters. Information on the nutritional requirement of fish and its availability from different sources is essential for formulation of complete feed. The present work describes the nutritional requirement for the development of fisheries and aquaculture of *Tor tor*. The main aim of the study is to find out which formulated feed is more beneficial to obtain a faster and better

growth rate of this particular fish species when cultured in captivity in tropical climatic conditions. It will help to evaluate the dietary requirements of the fry to adult stage of *Tor tor*, so as to formulate nutritionally balanced diets.

Materials and methods

Growth is a useful integrated index of the physiological status of an organism. The present study was carried out from July 2006 to June 2007, in 2 experimental trials, to evaluate the change in growth of *Tor tor* during its rearing on various feeds. The experiments were conducted in order to identify suitable formulated feed having different protein contents for rearing mahseer (*Tor tor*), ranging from fry to advanced fingerling stage, in captivity in tropical climatic conditions, and the results were compared with the natural conditions.

Treatments

T-1: Fish fed with feed containing 25 % protein level
T-2: Fish fed with feed containing 35 % protein level
T-3: Fish fed with feed containing 40 % protein level
T-4: Fish fed with the available commercial feed (ACF) {Crude Protein (CP) = 20 %)}
For evaluating the growth performances, the following methodology was used.

Methods for the study of fish growth

Net length/weight increment

The net length and weight of fish was calculated as under;

Calculations: Net increment in length = L2 – L1
Net increment in weight = W2 – W1

where L1 = Length of fish at time T1 (Initial time)
L2 = Length of fish at time T2 (Final time)
W1 = Weight of fish at time T1 (Initial time)
W2 = Weight of fish at time T2 (Final time)

Percent length/ weight increment

The percentage increment in the length and weight of fish was calculated by employing the following formula;

$$\% \text{ Increment in length} = \frac{L2 - L1 \times 100}{L1}$$

$$\% \text{ Increment in weight} = \frac{W2 - W1 \times 100}{W1}$$

Per day length/weight increment

The per day increment in length/weight of fish was calculating by employing the following formula.

$$\text{Per day increment in length} = \frac{L2 - L1}{T2 - T1}$$

$$\text{Per day increment in weight} = \frac{W2 - W1}{T2 - T1}$$

Data analysis for FCR, SGR and PER- the following formulae were employed;

$$\text{Feed conversion ratio} = \frac{\text{Amount of dry feed consumed}}{\text{Live weight gain}}$$

$$\text{Specific growth rate} = \frac{\text{Log final body weight} - \text{log initial body weight}}{\text{Number of days}} \times 100$$

$$\text{Protein efficiency ratio} = \frac{\text{Gain in body weight}}{\text{Protein intake}}$$

Length-Weight relationship

The general parabolic equation of the form $W = aL^3$ [6] which explains the length-weight relationship in fish, was used in the present study. This equation was written in the linear form as $Y = a + bx$, where Y = Log W, and x = Log L. Hence, 'a' and 'b' were estimated empirically by the method of least square.

Preparation of formulated feeds

In the present study, for preparing feed, the main ingredients used were fishmeal, soybean cake, ground nut oil cake and soya oil as a protein source, wheat bran and rice bran as a carbohydrate source, and molasses, mineral mixture and tapioca as a binder (**Table 1**).

Table1 Formulation of 3 different diets and an available commercial feed (1 kg) for *Tor tor* with different protein levels.

Ingredients	Experimental Diets (%)			ACF
	Feed I (25 % P)	Feed II (35 % P)	Feed III (40 % P)	ACF (20 %)
	(g)	(g)	(g)	(g)
Fishmeal	200	350	400	050
Soya cake	400	400	350	100
GNOC	200	050	-	-
Wheat bran	050	050	100	500
Rice bran	050	050	050	250
Soya oil	020	020	020	-
Molasses	060	060	060	080
Mineral mixture	020	020	020	020

Available commercial Feed (ACF); GNOC: Groundnut oil cake
The formulated feeds were prepared at the Central Institute of Agricultural Engineering at Bhopal (CIAE).

Table 2 Mean growth performance, feed and nutrient efficiency of *Tor tor* fed various protein rations for 365 days.

Diet No.	T-1	T-2	T-3	T-4	SEM
Parameters					
Initial length (mm)	44.5	44.5	44.5	44.5	22.25
Final length (mm)	98.5±3.5	111.5±2.6	114.3±3.1	78.6±2.5	8.13±1.2
Percent length increment	121.34±5.4	150.56±6.2	156.85±5.8	76.62±3.2	18.29±2.1
Initial weight (g)	2.102	2.102	2.102	2.102	0
Final weight (g)	6.317±1.1	6.629±1.02	7.136±1.2	2.746±0.5	1.001±0.06
Percent weight increment	200.52±12.2	215.36±8.9	239.48±6.7	30.63±3.5	47.63±3.9
Initial per day length (mm)	0.153±0.02	0.173±0.05	0.187±0.04	0.12±0.05	0.014±0.03
Final per day length (mm)	0.09±0.001	0.15±0.002	0.175±0.001	0.055±0.002	0.027±0.001
Initial per day weight (g)	0.014±0.001	0.019±0.002	0.02±0.001	0.004±0.001	0.003±0.002
Final per day weight (g)	0.009±0.001	0.011±0.00	0.015±0.001	0.003±0.00	0.0025±0.001
Specific growth rate (g % Day $^{-1}$)	0.130±0.002	0.136±0.002	0.145±0.002	0.0317±0.001	0.026±0.001
Food conversion ratio	1.3±0.02	1.12±0.03	0.82±0.02	1.8±0.03	0.21±0.01
Protein energy ratio	0.428±0.02	0.585±0.01	0.846±0.02	0.083±0.02	0.159±0.01

Note: Values are means±SD of 3 replications (d.f. 4, 35).
Initial per day length and final per day length are the measurements of length in morning and evening.
Initial per day weight and final per day weight are the measurements of weight in morning and evening.

Feed preparation

All the locally available ingredients were dried in a hot air oven at 60 °C for 24 h. All the ingredients were ground into a powdered form by an electrically operated grinder. The binder (tapioca) was also ground in an electrically operated grinder before preparation of pellets.

Feed formulation

Feed formulation was done basically by using the "square method" in order to initially balance the basal feed and protein supplement. The proportion of each ingredient required was calculated precisely, providing allowance for the premix [7].

Results and discussion

Results

The results of the present findings revealed that *Tor tor* fry with an average length of 44.5 mm and a weight of 2.102 g, subjected to 3 formulated diets containing 25 % protein (T-1), 35 % protein (T-2) and 40 % protein (T-3), showed considerable changes in growth parameters in contrast to T-4, in which the fish were subjected to available commercial feed (ACF) containing 20 % of crude protein. The other feed constituents were the same as that of the commercial feed, and the main focus was to assess the feed preference of *Tor tor* in response to a change in protein concentration.

Table 2 depicts the results for the growth of *Tor tor*. Out of the 4 treatments, T-3 showed the maximum increase in length (mm) (114.3±3.1) after the stipulated research tenure of 12 months (365 days). The lowest increase was shown by T-4 (ACF), with a maximum length (mm) of 78.6±2.5. The mean±SD for the treatment groups was calculated as 8.13±1.20. ANOVA (**Table 3**) for the total length (*df* = 35) attainment among treatment groups showed no significant relation ($P > 0.05$) among the treatment groups. However, significant differences ($P < 0.05$) were observed in percent length among the treatment groups, calculated mathematically. In the case of final weight, the highest weight (gms) was achieved by fish subjected to T-3 (7.136±1.2) in comparison to the ACF group (T-4), which showed a final weight of 2.746±0.5. The mean±SEM for the treatment set was 1.001±0.06. Significant relation ($P < 0.05$) was recorded among the treatments groups (*df* = 35). A similar trend was observed for per day length and per day weight, corresponding to the attainment of final length and weight at the end of the experiment. ANOVA (**Table 3**) showed significant ($P < 0.05$) relations among the treatment groups (*df* = 31) in case of per day length and per day weight.

The Specific Growth Rate (SGR) analysis revealed the highest SGR value 0.145±0.002 in the case of treatment 3, as compared to fish subjected to ACF. The mean±SEM for the treatment set was 0.026±0.001. ANOVA in the case of SGR revealed significant differences ($P < 0.05$) among the treatment groups (*df* = 31). The lowest FCR (0.82±0.02) was observed in T-3 (40 % CP), as compared to the other two high protein treatments. The highest FCR (1.8±0.03) was recorded for T-4, which received feed containing 20 % CP. The mean±SEM for the treatment set was 0.21±0.01. ANOVA for FCR revealed no significant differences ($P > 0.05$) among the treatment groups (*df* = 31). In the case of PER, the highest value (0.846±0.02) was recorded in T-3 (40 % CP), as compared to ACF with PER as low as 0.083±0.02. The mean±SE for the treatment set was 0.159±0.01. ANOVA for SGR revealed significant difference ($P < 0.05$) among treatment groups (*df* = 31). The variance among the treatment groups is illustrated in **Table 3**.

The length weight relationship for T-1 (CP = 25 %) was calculated as $y = 0.073x - 1.314$, with an 'r' value of 0.988. In T-2 (CP = 30 %), the 'a', 'b' and 'r' values were calculated as –0.693, 0.064 and 0.997 respectively. In the case of T-2 (CP = 40 %), the regression equation was calculated as $y = 0.067x - 0.774$, with 'r' value of 0.996. In a set of fishes fed with a normal commercial diet (CP = 20 %), the 'a', 'b' and 'r' values were calculated as 1.620, 0.012 and 0.92 respectively (**Figures 1 - 4**).

Table 3 ANOVA of different growth parameters of *Tor tor* fed with varying concentrations of protein.

S.N.	Parameter	*df*	SS	MS	F	F Crit	p-value	Variance
01	Length (mm)	35	11326.98	206.08 (WG) 334.64 (BG)	0.615	2.901	0.609	T-1 = 287.7 T-2 = 447.7 T-3 = 475.9 T-4 = 127.2
02	Weight (g)	35	64.21584	5.533648 (WG) 1.487965 (BG)	3.718	2.901	0.02	T-1 = 1.641 T-2 = 1.902 T-3 = 2.372 T-4 = 0.034
03	Per day length (mm)	31	0.09055	0.016146 (WG) 0.001504 (BG)	10.73	2.946	0.00	T-1 = 0.000 T-2 = 0.002 T-3 = 0.001 T-4 = 0.001
04	Per day weight (g)	31	0.001205	0.000185 (WG) 0.0000023 (BG)	7.97	2.946	0.0005	T-1 = 0.000 T-2 = 0.000 T-3 = 0.000 T-4 = 0.000
05	SGR	31	1.047468	0.103155 (WG) 0.026357 (BG)	3.913	2.946	0.018	T-1 = 0.006 T-2 = 0.010 T-3 = 0.010 T-4 = 0.003
06	FCR	31	325.12	4.832752 (WG) 9.70712 (BG)	0.497	2.901	0.686	T-1 = 10.13 T-2 = 7.122 T-3 = 3.753 T-4 = 17.81
07	PER	31	71.42248	6.587857 (WG) 1.844961 (BG)	3.5707	2.946	0.026	T-1 = 1.145 T-2 = 2.429 T-3 = 3.044 T-4 = 0.759

SGR = Specific growth rate; FCR = Food conversion rate; PER = Protein energy ratio

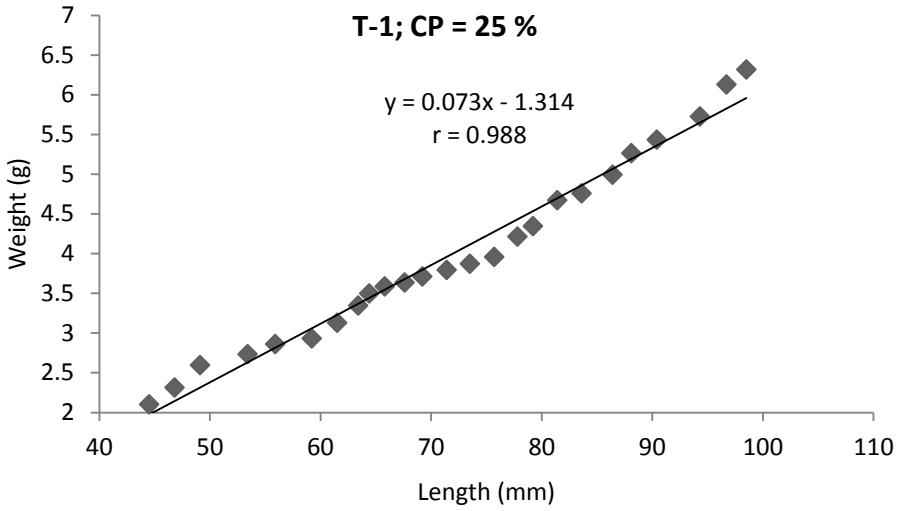

Figure 1 Length-weight relation for different protein rations fed to *Tor tor* fry at T-1; CP = 25 %.

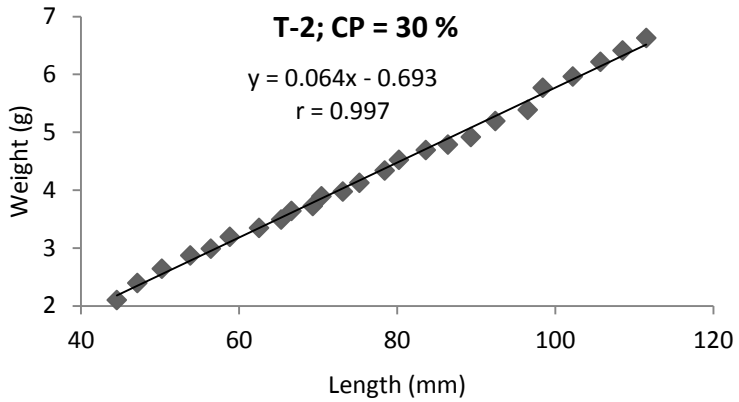

Figure 2 Length-weight relation for different protein rations fed to *Tor tor* fry at T-2; CP = 30 %.

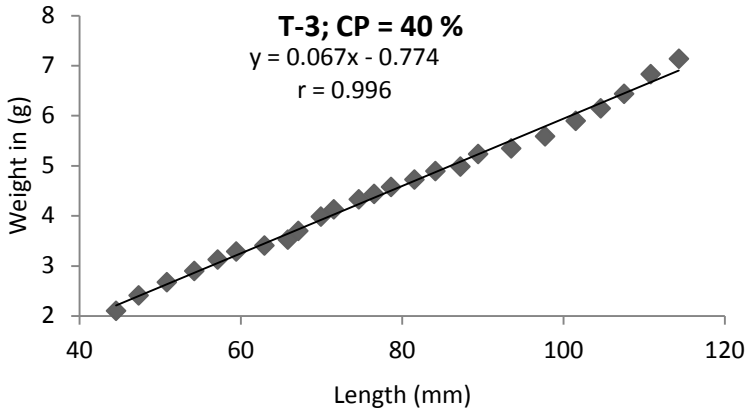

Figure 3 Length-weight relation for different protein rations fed to *Tor tor* fry at T-3; CP = 40 %.

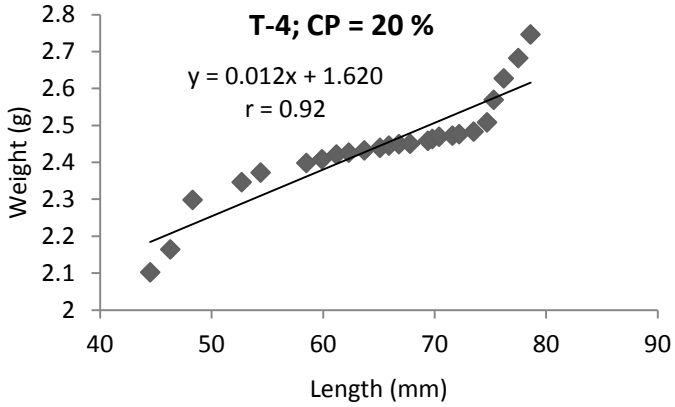

Figure 4 Length-weight relation for different protein rations fed to *Tor tor* fry at T-43; CP = 20 %.

Discussion

The present research work was an effort made with an intention to upgrade aquaculture nutrition science, with respect to commercially important fish such as *Tor tor* [2], by using different protein ratios for determination of a feed formulation which would reduce the FCR value and make fish growth economical. There have been numerous attempts to formulate the diets as per the requirement of the fish. This work is no exception to the previous attempts, barring that it encompasses a featured work on all the aspects and feed formulation, feeding and its impact on various physio-biochemical aspects of the test species. The work can be used by future researchers and will be a commendable contribution to aquaculture nutrition.

A feeding trial was conducted to investigate the effects of dietary protein levels on growth, feed utilization, hepatosomatic index and liver lipid deposition of juvenile red snapper, *Lutjanus argentimaculatus* [8]. In their experiment, 6 fishmeal-based diets were formulated to contain various protein levels (20 % to 45 % in 5 % increments). The fish at the end of the study had a more than 10-fold (77.0 g) increase in weight compared to the initial weight (8.0 g). Fish fed diets of 40 % and 45 % protein produced significantly ($P < 0.05$) higher weight gains of 77.2 and 76.5 g, and specific growth rates (SGR) of 2.65 and 2.62 %. The studies of the above author correspond to the results of the present study, which showed an increase in weight (7.136±1.2) and SGR (0.145±0.002) in *Tor tor* fed on 40 % CP diets.

Effects of the protein/carbohydrate ratio of extruded diets on protein synthesis, protein growth and body composition in juvenile brown trout (*Salmo trutta*) [9]- the authors reported differences in specific growth rates (C: 0.88 %; HC: 0.77 %), associated more with the lower protein consumption rate and the lower level of digestible energy in fish fed HC than with the higher dietary carbohydrate content of the diet, documenting the impact of high protein on overall increase in SGR and PER. Tabassum and Mukhtar [10] worked on the effects of dietary protein levels on growth, feed utilization, protein retention efficiency and body composition of young *Heteropneustes fossilis* (Bloch). The results of our research get complete support from the work of Tabasum and Mukhtar [10].

An 8-week growth study was conducted to determine the effect of ration level, energy, and protein maintenance requirement of catfish, *Heteropneustes fossilis* (Bloch), fingerling (7.90±0.55 cm; 3.10±0.28 g) by feeding them a casein-gelatin-based purified diet (40 % CP; 3.61 kcal g-1 GE) at 6 ration levels, 1 - 6 % of BW/day [11]. The authors concluded that the maximum live weight gain, best feed conversion ratio (FCR), best specific growth rate (SGR), and highest protein efficiency ratio (PER) were evident for ration levels of 45 % body weight (40 - 45 % CP). This is in complete agreement with the findings of the present study. In 2011, Nilnaj *et al*. [12] worked on the effects of dietary protein and lipid levels and protein to energy ratios on growth performance and feed utilization of hatchery-reared juvenile spotted babylon (*Babylonia areolata*). A 120 day feeding trial was designed by the authors to determine the effects of different dietary protein and lipid levels and protein to energy ratio (P:E). Six diets were formulated to contain three protein levels (18, 28 and 36 %). The results showed that the highest ($P < 0.05$) values for growth and feed efficiency were observed for snails fed a diet containing a 36 % protein level, and the same trend was observed for snails fed a diet with a 10 % lipid level. The higher protein levels substantiated by the appropriate energy contents increased the growth coefficient, as evidenced in the present study and documented by the above authors.

Seemab and Khan [13] enumerated the dietary protein requirement for fingerlings of *Channa punctatus* (Bloch), based on growth, feed conversion, protein retention and biochemical composition. The authors reported that the maximum absolute weight gain (AWG; 8.11 g $fish^{-1}$), specific growth rate (SGR; 1.82 %) and best feed conversion ratio (FCR; 1.48) were recorded in fish fed diet containing 450 g kg^{-1} protein, whereas the protein efficiency ratio (PER; 1.52), protein retention efficiency (PRE; 25 %), energy retention efficiency (ERE; 78 %) and RNA/DNA ratio (3.01) were maximum for the group fed dietary protein at 400 g kg^{-1}. The work of the above authors lends complete support to the present findings, stating that with the appropriate incorporation of energy source, the protein proportion could be utilized as the best convertible food substance by the mahaseer *Tor tor*.

Conclusions

The present study revealed that the inclusion of higher levels of protein (CP = 40 %) in Mahaseer (*Tor tor*) diets, containing an appropriate P:E ratio, is feasible for the better growth and food conversion efficiency of the given feed. As such, research in the field of Mahaseer nutrition needs some more information to make the dietary needs of *Tor tor* more understandable.

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