Biochemical changes in proximate and body composition of *Tor tor* (Hamilton, 1822) fed with various concentrations of protein

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Abstract

The present study was aimed to assess the proximate biochemical composition of body and faecal matter of *Tor tor* fed with feed combination containing 25% CP (T-1), 30% CP (T-2), 40% CP (T-3) and control group (T-4) containing 20% CP. The results of the proximate composition of the faeces of *Tor tor* revealed the higher CP% of 18.64±0.03 in fishes fed on feed containing 40±0.01 CP in the basal diet. The whole body carcass analysis revealed higher concentrations of accumulated CP% as high as 65.34±34 (T-3), fed with 40% basal crude protein. The overall study revealed that inclusion of 40% CP in mahaseer diet could lead to better growth and feed efficiency.

Key words: Crude Protein, *Tor tor*, Carcass composition, Biochemistry

Introduction

Tor tor is the most common Himalayan mahseer and a very attractive sport fish with excellent food value. *Tor tor* is a highly nutritious fish with good economic value. It shows a steady decline in abundance in reservoirs in India. It inhabits riverine pools and lakes and also in 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 ^{o}C) at depths of upto 15 m. Adults have an 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 (Desai, 2003). Among Indian mahseers, *Tor tor* (Hamilton, 1822) is the most important food and game fish of India after *Tor putitora* (Hamilton, 1822).

Studies on the nutritional aspects from 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 NRCCWF, while in case of khudree mahseer more systematic studies have been conducted to evaluate the optimum protein requirement (Murthy and Keshavanath, 1986), protein sources (Keshavanath *et al.*, 1986) and protein sparing effect of sardine oil (Bazaz and Keshavanath, 1993). 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 so as to increase its 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 with optimum protein level is more beneficial to obtain fast and better growth rate of this particular fish species when cultured in captivity in tropical climatic conditions. It will help to evaluate dietary requirement of fry to adult stage of *Tor tor*, so as to formulate nutritionally balanced diets.

Materials and Methods

Treatments

T-1: Fishes fed with feed containing 25% protein level
T-2: Fishes fed with feed containing 35% protein level
T-3: Fishes fed with feed containing 40% protein level
T-4: Fishes fed with the available commercial feed (ACF) containing 20% CP

1. Proximate analysis of feed ingredients and feeds.

Proximate analysis of all the formulated feeds was performed according to AOAC (1995) methods. The feeds were analyzed before formulation of different diets.

1.1. Estimation of moisture content

Moisture content was estimated by the following formula

$$\% \text{ moisture} = \frac{\text{Sample wt. (g)} - \text{Dried sample wt. (g)}}{\text{Sample wt. (g)}} \times 100$$

1.2. Estimation of Ash content

Ash estimation was calculated as under:

$$\% \text{ Ash} = \frac{\text{Ash weight (g)}}{\text{Sample weight (g)}} \times 100$$

1.3. Estimation of Crude Protein content

The estimation of crude protein content involved three phases i.e, digestion, distillation and titration. The crude protein was calculated by the formula:

$$\% \text{ Crude protein} = \frac{V \times 0.00014 \times D \times 100}{W \times A} \times 6.25$$

where, V = Volume of N/100 H_2SO_4
D = Dilution factor
W = Weight (g) of sample
A = Aliquot taken

1.4. Estimation of Crude Lipid content

Crude lipid content was calculated by the formula

Calculation:

$$\% \text{ Lipid} = \frac{\text{Cup weight after extraction (g)} - \text{Empty cup weight (g)}}{\text{Sample weight}} \times 100$$

1.5. Estimation of Calcium content:

0.5 g of ash sample was taken in a Kjeldahl tube and 20 ml of concentrated

HNO_3 was added to it. The solution was then digested in a fume cupboard at 110^oC till it became clear. Simultaneously 3 numbers of blank (digestion tube + acid) were digested. The volume was made to 50 ml by adding distilled water and stored in polythene bottles till analysis was done by atomic absorption spectrophotometer following Factar's instructions.

1.6. Determination of Gross energy:

Gross energy was calculated by the formula:

$$\text{Calculation:} \quad \frac{(\text{Rise in temperature} \times 3147) - 23}{\text{Sample weight (g)} \times 1000}$$

(Combined energy value of nickel wire + cotton = 23 Cal).

1.8. Percentage of feed

The experiment had four dietary treatments, three prepared formulated feeds and one commercial feed. The fry's kept in three tanks (aquarium) during July 2005 to June 2006 (first trial) were fed on feeds having 25%, 35% and 40% protein levels and the fourth tank (aquarium) fry's were fed on commercial feed having 20% protein content. The feeding was done daily @ 5% body weight. The feed was given twice daily morning and evening.

1.9. Collection of wasted feed (Wf) and faecal matter (Fm) for proximate analysis.

To determine the proximate composition of faeces, it was collected at different times from the tanks fed with different diets. This was done for a number of days till the quantity was sufficient for analysis. The faecal and wasted feed was collected by siphon pipe and dried in an oven in a petridish. The collected matter from respective tanks were pooled together and stored in air tight bottles until analysis. Same methods were used for proximate analysis.

1.10. Proximate analysis of body carcass.

At the start and end of both the experiments the fishes were taken and their total length and body weights were recorded. After evisceration, the fishes were cut into pieces. These collected pieces were weighed and put into hot air oven at 60^oC for 72 hours for drying to determine the moisture content. The dried samples in aluminium foil were stored in dessicator for proximate analyses following the procedure as described for feed.

Results

The present study was aimed at the assessment of the biochemical constituents of fish food containing 25% CP (T-1), 30% CP (T-2), 40% CP (T-3) and control group 20% CP, fed to *Tor tor* fry and assessment its effect on the biochemistry of faecal matter and carcass at the end of the experimental period. The study shall be a valuable information tool for assessment of the protein inclusions into the feed and its subsequent effect on biochemistry of body composition of the fish. The percentage composition of four feed treatments is presented in table 1. The biochemical constitution of the formulated feed showed that the CP concentration was higher in T-3 (40.0±0.01). The treatment group had the appropriate energy content as higher levels of crude lipid (19.14±0.12) and carbohydrate (38.78±1.01). The gross energy

(Kj/g) for the treatment group was enumerated to be 17.28±1.21. In comparison to other groups which included less P:E ratio showed gross energy as 16.48±0.34 (T-3; CP = 30%) and 15.82±0.21 (T-2; CP = 25%). The routine feed (ACF) which is fed to Mahaseer (*Tor tor*) contained CP (%), lipid (%) and energy content (Kj/g) of 20.0±0.01, 17.23±0.10 and 13.51±0.12 respectively.

Table 2 demonstrates the proximate composition of waste feed and faecal matter of *Tor tor*. The waste feed and faecal matter was collected on routine basis daily and subjected to biochemical analysis. The results are the mean±SD of the results. Among four treatments, the Cp content was highest in T-3 (5.25±0.11), as compared to the ACF, which contain CP (%) as less as 2.14±0.07. The lipid content was recorded as 3.16±0.06 for T-3, with as less as 1.14±0.04 (ACF) and as high as 5.37±0.01 (T-1). The gross energy (Kj/g) analysis of the feed and waste material revealed minor differences among the treatment groups, with T-1 having the highest gross energy (7.61±0.02) and T-4 (ACF) exhibiting the lowest GE of 5.9±0.04. The initial and final biochemical components of the body carcass of fish subjected to various dietary protein levels ate presented in table 3. The initial CP (%) value (59.67±0.08) increased to the value of 65.34±0.10 in T-3, which was the highest among the other treatment groups. In case of ACF, the CP (%) showed a minor increase with a value of 58.78±0.10. The proximate analysis of crude lipid (%) in T-3 showed an increase to the tone of 25.27±0.01 from the initial value of 17.57±0.02. Besides other components like ash (%) and dry matter (%), gross energy (Kj/g) showed a remarkable increase to the tone of 20.78±0.01, as compared to the initial value of 16.32±0.01. Overall the biochemistry of feed, unfed feed, faecal matter and the body carcass revealed an enormous change subjected to the basic feed formula which mainly utilized the inclusion of 40% CP, added by the adequate sources of energy.

Discussion

The present research work is an effort put with an intention to upgrade the aquaculture nutrition science, with respect to commercially important fish (Mahaseer), by using different protein, lipid and carbohydrate ratios for determination of a feed formulation, which would reduce the FCR value and makes the fish growth economical. Although there has been so many attempts to formulate the diets as per the requirement of the fishes, present work is no exception to that, except 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 as an engender for the future researchers and will be a commendable contribution to aquaculture nutrition.

The biochemical composition of various food ingredients used as food for fish and shrimp has been thoroughly authenticated by FAO. In order to get an appropriate idea regarding the performance of a feed at all metabolic levels, the biochemical composition of the formulated diet needs to be charted out. It is better to change the feed composition for Mahaseer, taking its omnivorous feeding habit into consideration, the main constituent being crude protein. In our results, the treatment 3 (CP = 40%) performed well at all biochemical levels. An attempt to determine the effects of four rations on growth, chemical composition and digestibility of the Rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) was done by Gumus and Ikis (2009). Four test diets were formulated to have the same levels of protein (40%) and energy (gross energy: around 4800 cal/g), however, these test diets were composed of basic diet with different carbohydrate (0, 3, 12 and 18%) to lipid (0, 6, 15 and 18%) ratios (Diet 1: 0/18; Diet 2: 3/15; Diet 3: 12/6 and Diet 4: 18/0). The authors observed that the percentage of water, protein and ash of fish flesh did not show any (P>0.05)

change. However, the muscle lipid content of fish significantly (P<0.05) decreased as carbohydrate level increased. The authors hypothesis supports our findings, which revealed protein assimilation while enumerating biochemistry of carcass.

Dietary protein and energy requirements of juvenile freshwater angelfish (*Pterophyllum scalare*) were evaluated by Zuanon, *et al*. (2009). The authors used 3 × 2 factorial design with three dietary crude protein levels being tested (26, 30, and 34% of CP) combined with two digestible energy levels (3,100 and 3,300 kcal DE/kg of diet) in three replicates. The authors observed that the fish fed diets with 26% CP showed greater protein efficiency values when compared to those fed diets with 34% CP. Diets with 26% of CP and 3100 kcal DE/kg could meet the nutritional requirements of juvenile freshwater angelfish. The dietary protein requirement for optimal growth performance and body composition of juvenile sole fish was evaluated by Abdel *et al*. (2011). Four diets were formulated with different protein levels (D40, D45, D50 and D55% crude protein). The authors used silverside fish (*Atherina boyeri*) as additive to the diets as local animal protein source and to enhance palatability of the test diets. Their results showed significant differences (P<0.05) in growth performance and feed efficiency between diets. No significance difference (P<0.05) in whole body chemical composition (dry matter, crude protein, crude lipid and ash) were found between fish fed all experimental diets. However, slight increase in whole body lipid contents were recorded with D50% CP and D55% CP levels. The results of the present study indicated that, D55% crude protein level can be optimal for meeting the requirement of juvenile sole, *Solea aegyptiaca* without adverse effects on growth performance and feed efficiency. The hypothesis of the above authors lends complete support to our findings that inclusion of higher concentrations of crude protein could lead to better feeding strategy

The present research findings of the inclusion of 40% CP in Mahaseer diets is completely supported by the work of Muzaffar *et al.* (2012 a,b) who used 40% CP in carp diet. The authrors reported the highest carcass dry matter, crude protein, crude lipid, ash and energy content, lowest moisture content and carbohydrate content in fingerlings fed with Feed B which contained 40 ± 0.21% protein, 9.31 ± 0.25% lipid and 10.08 ± 0.10% carbohydrate, which supports our research findings. The effects of diets, containing different dietary protein levels (32, 36, 40, and 44%), on growth, feed conversion rate and survival of slender walking catfish (*Clarias nieuhofii*) fingerlings were studied by Suphada and Anut (2012). Fishes were fed with isocaloric test diets containing 32, 36, 40 and 44% protein, for 12 weeks. Carcass composition analysis indicated a positive correlation between dietary protein level and fish body protein content, but moisture, lipid level and ash content in the fish body were not significantly different among treatments. In conclusion, the 40% protein diet gave the maximum growth performance, lowest feed conversion ratio and high body protein content in slender walking catfish fingerling during the 12 weeks of the feeding trial. The findings of Suphada and Anut (2012) lend complete support to our findings.

Conclusion

The present study revealed that the mahaseer culture in captivity, if fed on a diet containing optimum levels of CP and appropriate level of DE, boosts the growth of the fish and makes it economically viable. The results of the proximate composition of the faeces of *Tor tor* revealed the higher CP% of 18.64±0.03 in fishes fed on feed containing 40±0.01 CP in the basal diet. The whole body carcass analysis revealed higher concentrations of accumulated CP% as high as 65.34±34 (T-3), fed

with 40% basal crude protein. The overall study revealed that inclusion of 40% CP in mahaseer diet could lead to better growth and feed efficiency

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