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Optimum Dietary Protein Requirement of Mahseer, *Tor putitora* (Hamilton) Fingerlings

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Abstract

The dietary protein requirement of fingerling mahseer, *Tor putitora* (Ham.) was investigated by feeding seven semi-purified diets containing varying levels of dietary protein within a range of 20 to 50%, using casein and gelatine as the dietary protein source, over a 50 day feeding period. The experiment was conducted in a static rearing system consisting of 14 glass aquaria of 55 l capacity. Each aquarium was stocked with 12 fingerlings of mahseer with a mean initial weight of 1.09 ± 0.002 g. Each treatment had two replicates and fish were fed twice daily at satiation level. The results of the study showed that the weight gain and specific growth rate (SGR % per day) of fish increased proportionally with the increase in dietary protein concentration to a level of 40% and thereafter, decreased with further increase in dietary protein levels. Fish fed on diet containing 40% protein level showed the significantly highest ($P<0.05$) weight gain. The feed conversion ratio (FCR) values ranged between 1.12 and 2.21. The protein efficiency ratio (PER) values ranged between 1.50 and 2.38 and the apparent net protein utilization (ANPU) values ranged between 24.69 and 32.24%. Apparent protein digestibility (APD) increased with the increase in dietary protein levels and ranged between 80.02 and 90.88%; diet containing 40% protein showed the highest APD value. There was progressive increase in carcass protein and a decrease in carcass lipid with progressive dietary protein increment. Based on observed growth performance, the dietary protein requirement of fingerlings of *T. putitora* was approximately 40%.

Introduction

Nowadays the mahseer, *Tor putitora* (Hamilton) is considered as a rare food fish and fetches a higher price than other fish in Bangladesh. This fish is an inhabitant of hilly streams, channels and tributaries and has a wide distribution in India, Nepal, Bangladesh and Pakistan (Chandra, 1977). Over the decades the natural population of this omnivorous species has declined due to the deterioration of environmental conditions and man-made pressure such as siltation and soil erosion on hilly rivers and reservoirs. Since natural stock of this fish is already in danger due to genetic erosion, mahseer is being considered as an endangered fish species in Bangladesh. To maintain or increase the population of this fish, conservation as well as the development of technology for breeding, rearing and nursery of fry and fingerlings are essential, either in culture or as stock in open water bodies.

Culture of *T. putitora* provides the country an opportunity to reduce protein deficiency and to earn foreign currency by exporting this fish. To intensify rearing and culture of this species, provision of nutritionally balanced rations becomes necessary. It is essential to know the minimum protein requirement for optimum growth in formulating a balanced ration since protein is an important major nutrient for growth and other metabolic activities, as well as costs more than carbohydrate and fat. Excess dietary protein not only costs more but also increases the energy cost of assimilation by increasing the specific dynamic action (LeGrow and Beamish 1986). Numerous investigators have utilized various semi-purified and purified diets to estimate the protein requirement of fish. Most of these values have been estimated from this level of protein yielding the minimum amount of dietary protein, which resulted to maximum growth. The dietary protein requirements of several species of young fish have recently been tabulated in NRC (1983). In general, the values range from about 30 to 55% crude protein for maximum growth.

The quantity of protein required by aquaculture animals varies with species, age, condition and reproductive state as well as variation in the environment (NRC 1983). Knowledge about the nutrient requirement of a species is a prerequisite in the formulation and development of a commercial diet. Since no published work is available so far on the dietary protein requirement of *T. putitora*, this experiment was conducted to determine the optimum dietary protein requirement of *T. putitora* under laboratory condition.

Materials and Methods

Experimental system

The experiment was conducted in a static indoor rearing system at the laboratory of the Department of Aquaculture, Bangladesh Agricultural University, Mymensingh for a period of 50 days during the months of May to June 1998. The experimental system consisted of 14 rectangular glass aquaria of 55 l capacity containing about 50 l of water used as the experimental tanks. Tap water was used in the aquaria during the experimental period. An adequate level of oxygen in each aquarium was maintained through artificial aeration. Natural photoperiod of 12h dark and 12h light was maintained throughout the experimental period.

Experimental fish and acclimation

Artificially bred fingerlings of mahseer (*T. putitora*) were collected from the Freshwater Station of Bangladesh Fisheries Research Institute, Mymensingh. The collected fish were given a prophylactic treatment with 0.5 ppm $KMnO_4$ solution for 30 min. During treatment, oxygen supply was maintained through artificial aeration. Fish were kept in a big plastic pool of 400 l before transferring to the experimental tanks. Before starting the experiment the fish were acclimatized to the experimental condition for one week. During this period, the fish were fed a diet containing 35% protein.

Experimental procedure and sampling

Seven treatments were scheduled for the experiment. Each treatment had two replicates; 12 fish per replicate with a mean initial weight of 1.09 ± 0.002 g. Fish were randomly distributed to the experimental tanks. Initial and final weights of the experimental fish in each tank were recorded individually using an electronic balance (Metler PM 480, Delta range). For intermediate weighing, fish were bulk-weighed every 10 days. Water in the aquaria was partially changed twice daily during the removal of feces by siphoning.

Experimental diets

Seven purified diets containing 20, 25, 30, 35, 40, 45 and 50% protein level were formulated to determine the dietary protein requirement of *T. putitora*. Casein and gelatine were used as protein sources since no quality (grade 1) fishmeal was available in the market. Prior to the formulation of diets, the protein sources were subjected to proximate analysis and the results are presented in table 1. The experimental diets were formulated to contain 20 to 50% protein and ∝-cellulose was used as bulking or filler material (Table 2). To study protein digestibility, formulated diets contained 0.5% chromic oxide. Diets were subjected to proximate analysis and the results are presented in table 3. Diets were prepared using 1 mm diameter pelleting machine (Hobart mixing machine, model A 200). The resultant pellets were then sun dried for two days and stored separately in airtight polythene bags in a deep freezer for further use.

Table 1. Proximate composition of protein sources used (% dry matter basis)

Protein sources	Dry matter	Protein	Lipid	Crude fiber	Ash	NFE[1]
Casein	92.0	94.6	0.6	0.3	2.5	2.0
Gelatin	95.0	97.3	0.4	0.5	1.0	0.8

[1]Nitrogen free extract calculated as 100% (moisture + protein + lipid + ash + crude fiber)

Feeding rate

Throughout the study period, the fish were fed experimental diets twice daily: at 900 h in the morning and at 1700 h in the afternoon to satiation levels. A record of supplied feed was kept to determine the FCR, PER and ANPU%.

Feces collection

For the study of protein digestibility of diets, feces were collected during the last two weeks of the experimental period. Any uneaten food or feces from each aquarium was carefully removed by siphoning about 30 min after the last feeding. Feces were collected by siphoning separately from each replicate tank before feeding in the morning. Collected feces were then filtered, dried in an oven at 60^0C and kept in airtight containers for subsequent chemical analysis.

Table 2. Formulation (%) of experimental diets

Ingredients	Diet 1 (20 % protein)	Diet 2 (25 % protein)	Diet 3 (30 % protein)	Diet 4 (35 % protein)	Diet 5 (40 % protein)	Diet 6 (45 % protein)	Diet 7 (50 % protein)
Casein	10.86	16.14	21.43	26.71	30.00	37.78	42.50
Gelatin	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Cod liver oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Vitamin[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral[1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Binder (CMC)[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dextrin	40.00	40.00	40.00	35.00	30.50	25.22	20.00
α-cellulose	21.64	16.36	11.07	10.79	10.00	10.00	10.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50

[1]According to Hossain and Jauncey (1989)
[2]Carboxymethyl cellulose (high viscosity)

Table 3. Analyzed proximate composition of the experimental diets (% dry matter basis)

Components	Diet No.						
	1	2	3	4	5	6	7
Dry matter	94.21	94.01	93.43	93.53	93.88	93.94	94.08
Crude protein	19.56	24.81	31.11	35.37	40.66	45.92	51.02
Lipid	9.32	10.20	9.58	9.76	9.95	9.96	10.11
Ash	4.60	4.32	4.21	4.65	4.13	4.60	4.35
Crude fiber	16.65	13.33	8.84	8.20	8.25	8.21	8.33
NFE[1]	49.87	47.34	46.26	42.02	37.01	31.31	26.19
Chromic oxide	0.51	0.52	0.49	0.51	0.51	0.49	0.50
Gross energy (kJ g^{-1})[2]	16.56	17.69	18.70	19.03	19.45	19.70	20.79
P/E Ratio[3]	11.81	14.02	16.63	18.58	20.90	23.30	24.54

[1]Nitrogen free extract calculated as 100 % (moisture + protein + lipid + ash + crude fiber)
[2]Gross energy calculated according to Jauncey and Ross (1982)
[3]Protein to energy ratio calculated as mg protein/kJ of total energy

Carcass composition

At the beginning of the experiment 24 fish from the stock were sacrificed and used for proximate composition analysis, which was considered as the initial carcass composition of fish. At the end of the experiment all the fish in each replicate were sampled for final carcass analysis. No fish mortality was observed during the experimental period.

Water quality

Water quality parameters such as dissolved oxygen (DO), ph and temperature were monitored and measured weekly throughout the experimental period using a meter (DO and temperature: YSI, model 58, USA; pH: Jenway, model 3020, U.K.). The water quality parameters monitored during the study period were: temperature (24.4 to 32.1^{0}C), pH (6.8 to 7.6) and DO (5.8 to 7.7 mg·l).

Analytical method

Feed ingredients, diets, feces and fish samples were analyzed for their proximate composition according to the standard procedure given in AOAC (1980). Chromic oxide was determined using the wet-acid digestion technique of Furukawa and Tsukahara (1966). Calculations of growth parameters were conducted according to Castell and Tiews (1980). Statistical analyses were performed by one way analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (Duncan 1955). Standard error (± SE) of treatment means was calculated from the residual mean square in the ANOVA.

Results

Analyzed composition of the experimental diets

The analyzed proximate composition of the experimental diets is shown in table 3. The protein content in different diets varied between 19.56 to 51.02%. The variation of protein levels in all experimental diets from the expected level were very small. The variations in lipid and ash contents among the different experimental diets were also minimal. However, there were variations in crude fiber contents in different experimental diets. Diets 1 and 2 contained higher levels (16.65 and 13.33% respectively) of crude fiber, which is due to addition of ∝-cellulose during feed formulation to adjust the protein levels. Energy contents varied from 16.56 to 20.79 kJg^{-1} between the experimental diets. The progressive increase in the energy content was due to the increased casein content in the diet. The protein to energy ratio varied between 11.81 to 24.54.

Acceptability of diets

Acceptability of the different experimental diets was judged based on a subjective behavioral assessment of the feeding responses. All fish were acclimated to the experimental diets within 1 to 2 days of the initiation of the feeding trial. The acceptability of all the diets was more or less similar. Fish were observed to feed actively throughout the trial and all the feed was consumed within 15 min of administration.

Growth

Growth responses of mahseer fingerlings fed the experimental diets are presented as initial and final mean weights, percentage weight gain and SGR (% per day) in table 4. The weight increments of fish by different diets during the experimental period are graphically shown in figure 1. In table 4 it can be seen that among the experimental diets, diet containing 40% protein exhibited the best growth response while diet containing 20% protein resulted in the poorest growth. The ranking of the diets is: 5 > 4 > 6 > 3 > 2 > 7 > 1. The SGR of fish ranged between 1.86 and 2.42 with diet 5 producing the highest SGR (% per day) while diet 1 produced the lowest (Table 4).

Table 4. Growth and feed utilization by *T. putitora* fed experimental diets

Parameters	Diet No.							
	1	2	3	4	5	6	7	±SE[2]
Mean initial weight (g)	1.10^{a1}	1.08^{a}	1.09^{a}	1.08^{a}	1.09^{a}	1.08^{a}	1.10	0.002
Mean final weight (g)	2.78^{e}	3.10^{cd}	3.15^{cd}	3.36^{b}	3.66^{a}	3.29^{bc}	3.04^{d}	0.059
Mean weight gain (g)	1.68^{c}	2.02^{cd}	2.06^{bcd}	2.28^{b}	2.57^{a}	2.24^{bc}	1.94^{d}	0.068
% Weight gain	152^{e}	187^{cd}	188^{cd}	211^{b}	235^{a}	207^{bc}	176^{d}	641
Specific growth rate (SGR % per day)	1.86^{d}	2.10^{c}	2.12^{c}	2.27^{b}	2.42^{a}	2.25^{b}	2.03^{c}	0.034
Food conversion ratio (FCR)	2.21^{d}	1.69^{c}	1.69^{c}	1.26^{b}	1.12^{a}	1.26^{b}	1.30^{b}	0.038
Protein efficiency ratio (PER)	2.31^{ab}	2.38^{a}	1.90^{c}	2.21^{b}	2.20^{b}	1.75^{d}	1.50^{c}	0.035
Apparent net protein utilization (ANPU %)	28.07^{c}	31.36^{ab}	29.05^{bc}	32.21^{a}	32.24^{a}	27.45^{c}	24.69^{d}	0.748
Apparent protein digestibility (APD%)	80.02^{d}	83.42^{c}	84.10^{c}	88.20^{b}	90.88^{a}	88.44^{b}	86.72^{b}	0.580

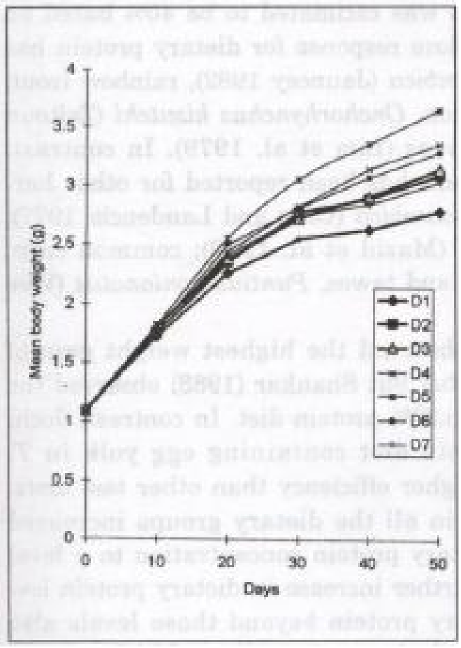
[1]Figures in the same row having the same superscripts are not significantly different (P>0.05)
[2]Standard error of treatment means calculated from the residual mean square in the analysis of variance

Feed utilization

The mean FCR of the different experimental diets ranged between 1.12 to 2.21 (Table 4). Diet 5 resulted in the lowest FCR i.e. the best FCR while diet 1 resulted in the highest i.e. the worst FCR. There were no significant differences between the FCRs of diets 4, 6 and 7 and 2 and 3 respectively.

The PER values ranged between 1.50 and 2.38 with diet 2 producing the highest and diet 7 the lowest PER values. However, there were no significant differences ($P > 0.05$) between the PER values of diets 1 and 2 and 1, 4 and 5 respectively.

The mean ANPU values ranged from 24.69 to 32.24% (Table 4). There were no significant differences ($P > 0.05$) between the ANPU values of diets 2, 4 and 5; 2 and 3 and 1, 3 and 6 respectively. The APD% values of the different experimental diets ranged between 80.02 and 90.88% (Table 4). There were no significant differences ($P > 0.05$) between the APD values of diets 4, 6 and 7 and 2 and 3 respectively.



Proximate carcass composition

Proximate carcass composition of fish at the start and at the end of the experiment are presented in table 5. Fish fed all the experimental diets had lower moisture content compared to that of the initial fish. Fish fed diets 3, 4, 5, 6 and 7 had higher protein contents compared to that of initial fish samples. The protein content was lower in low protein diet than in

Fig. 1. Weight increment of *T. putitora* fed the experimental diets during the study period

Table 5. Proximate carcass composition of experimental fish at the start and at the end of the experiment (% fresh matter basis)

Parameters	Final (Mean values) Diet No.							
	Initial (all fish)	1	2	3	4	5	6	7
Moisture	74.97	73.24	73.45	73.00	72.57	72.10	72.30	73.10
Protein	14.00	13.48	13.72	14.65	15.57	15.72	15.73	14.92
Lipid	6.73	9.60	9.01	8.72	7.40	7.51	7.39	7.21
Ash	3.34	3.22	3.53	3.56	4.29	4.53	4.39	4.53
Total	99.04	94.52	99.79	99.93	99.79	99.86	99.81	99.76

high protein diets (1 and 2 < 4, 5 and 6). In general, fish fed the experimental diets had higher lipid content compared to the initial and ranged between 6.73 and 9.60%. The lipid content of carcass was higher in the low protein diet than in the high protein diet (1, 2 and 3 > 4, 5, 6 and 7). The ash content ranged between 3.22 and 4.53%.

Discussion

The dietary protein requirement of various cultured fish species have been investigated by a number of authors (Lim et al. 1979, Jauncey 1982, Wee and Tacon 1982, Wee and Ngamsnae 1987, Santiago and Reyes 1991, Singh and Bhanot 1988) and these studies showed that the dietary protein requirement for fish varied from species to species due to feeding habit, size and water temperature.

In the present study, the minimum level of dietary protein, which exhibited maximum growth in *T. putitora* was estimated to be 40% based on weight gain, SGR and FCR. A similar dose response for dietary protein has been reported for tilapia, *Tilapia mossambica* (Jauncey 1982), rainbow trout, *Salmo gairdneri* (Satia 1974), coho salmon, *Onchorhynchus kisutchi* (Zeitoun et al. 1974), and milkfish, *Chanos chanos* (Lim et al. 1979). In contrast, slightly lower dietary protein requirement has been reported for other herbivorous species such as tilapias, *T. mossambica* (Cruz and Laudencia 1977); *T. nilotica* (Wang et al. 1985); *T. zilli* (Mazid et al. 1979); common carp, *Cyprinus carpio* (Ogino and Saito 1970) and tawes, *Puntius gonionotus* (Wee and Ngamsnae 1987).

Shyma and Keshavanath (1993) observed the highest weight gain of mahseer, *T. khudree* fed a 40% protein diet but Shankar (1988) observed the best growth rate of *T. khudree* with a 35.29% protein diet. In contrast, Joshi et al. (1989) reported that 35% protein diet containing egg yolk in *T. putitora* showed better results with a higher efficiency than other test diets.

The weight gain of fish and SGR in all the dietary groups increased proportionately with an increase in dietary protein concentration to a level of 40% and thereafter, decreased with further increase in dietary protein levels. The apparent digestibility of dietary protein beyond those levels also started to decrease. This apparent growth depressing effect of high protein diets observed in this study has also been reported for other fish species, such as snakehead (Wee and Tacon 1982), grouper (Teng et al. 1978), tilapia (Jauncey 1982) and grass carp (Dabrowski 1977). It has been postulated that the decrease in growth response at protein levels above the optimum may be due to the reduction in dietary energy available for growth as extra energy is required to deaminate and excrete the excess amino acids absorbed (Jauncey 1982). Lim et al. (1979) also reported that the slightly lower weight gain of milkfish, *C. chanos* (Forskal) fed diets with protein levels above the optimal could be due to insufficient non-protein energy in the diets. It has been suggested that diets with high levels of protein and low amounts of non-protein energy may be toxic to channel catfish, *Ictalurus punctatus*

(Prather and Lovell 1973). However, in the present study the contribution of non-protein energy (energy from lipid and dextrin) to diets 1 to 5 could be considered sufficient. The contribution of non-protein energy in diets 6 and 7 were comparatively lower and which might have affected the growth. If a diet contains enough non-protein energy yielding nutrients (fat and CHO), there would be less use of protein for energy purpose, hence the reduction in catabolism of protein and less non-fecal nitrogen loss.

The FCR values ranged between 1.12 and 2.21. There was a distinct trend for FCR to decrease with the increasing dietary protein levels, similar to the trend observed in tilapia (Jauncey 1982) and *P. gonionotus* (Wee and Ngamsnae 1987). However, the FCR values obtained in the present study were much lower than those reported for *P. gonionotus* by Wee and Ngamsnae (1987).

The PER in the present study followed a similar trend with that of the FCR and ranged from 1.50 to 2.38 with the highest value in fish fed the diet with 25% protein. In general, the PER values decreased progressively as the percentage of protein increased with the exception of diet 3 containing about 31% protein. The decreasing trend of PER was also observed with snakehead (Wee and Tacon 1982), grass carp (Dabrowski 1977) and *P. gonionotus* (Wee and Ngamsnae 1987). Fish often show the greatest protein conversion efficiency when fed dietary protein concentration less than that yielding the maximum growth and feed efficiency (Davis and Stickney 1978). The highest PER and ANPU% obtained in this study with *T. putitora* were found with 25% protein in the diet, the second lowest dietary protein concentration. The decreasing trend of ANPU values with increasing dietary protein concentration was also reported by Jauncey (1982) in tilapia and Wee and Ngamsnae (1987) in *P. gonionotus*. Steffens (1981) also reported that raising the dietary protein level improves the growth rate and food conversion but reduces PER and protein productive value (PPV).

The APD values ranged between 80.02 and 90.88%. There was a progressive increase in APD values with an increase in dietary protein levels up to 40%, thereafter it decreased. The highest APD value (90.88%) obtained with diet 5 (containing 40% protein) is similar to the true protein digestibility values (89.76%) reported by Jauncey (1982) for tilapia fed diet containing 40% protein. The low APD values obtained with low dietary protein levels may be due to the deleterious effect of high carbohydrate content of these diets (Shimeno et al. 1979, Singh and Nose 1967).

The dietary protein level had an effect on the proximate carcass composition. In general, there was a progressive increase in carcass protein and a decrease in the carcass lipid with progressive dietary protein increment. A similar trend has also been reported by various authors with common carp (Zeitter et al. 1984), tilapia (Jauncey 1982), silver barb (Wee and Ngamsnae 1987) and snakehead (Wee and Tacon 1982).

The importance of protein level in relation to the energy levels of the diets in fish is well recognized. The result of the present study showed that 40% protein with 19.45 kJ·g gross energy respectively are the best protein energy ratio (20.9) based on the growth and feed utilization of *T. putitora*.

This may be due to the fact that the protein level (40%) in diet 5 was optimum to promote growth and the energy supplied by this diet was also adequate for maintenance and growth. Hossain et al. (2001) observed best growth performance and feed utilization in common carp fed a fishmeal based standard diet with a similar protein energy ratio of 21.2.

In the present study, although all groups of fish were fed actively, comparatively poor growth response particularly during the later part of the experiment was observed. This could be due to the higher temperature that prevailed during the later part of the experimental period as well as the type of feeding regime (two times) employed. Fish fed with diet 5 (40% protein) only grew from 1.09 to 3.66 g in 50 days. Rai and Bista (1994) reported that *T. putitora* fry grew from 0.031 to 0.34 g after 40 days of rearing in cages set in earthen ponds. *T. putitora* is reported to spawn during the months of December to January when the water temperature is between 15 to 25^0C. The fish showed better growth performance within the water temperature range 20 to 24^0C (M.A. Hossain, BFRI, pers. com.). Although no published reports are available on the optimum temperature for their growth; it is assumed that a temperature above 30^0C may not be suitable for its growth. In other dietary protein requirement studies in which semipurified diets were fed to fish reared in clean water systems, relatively poor growth performances were also obtained in tilapia (Jauncey 1982), milkfish (Lim et al. 1979) and *P. gonionotus* (Wee and Ngamsnae 1987).

In the present study, the optimum dietary protein level for fingerling mahseer, *T. putitora* is determined to be about 40% based on growth performance, feed utilization and tissue protein deposition when casein and gelatine are used as protein sources.

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