Animal Nutrition and Feed Technology (2012) 12 : 179-186

Effect of Feeding Different Levels of Protein on Growth Performance, Feed Utilization and Digestive Enzyme of Grey Mullet (*Mugil Cephalus* L)

Debasis De*, T.K.Ghoshal and Joydeep Kundu

Kakdwip Research Centre of Central Institute of Brackishwater Aquaculture
Kakdwip, West Bengal- 743347, India

(Received July 12, 2011)

ABSTRACT

De, D., Ghoshal, T.K. and Kundu, J. 2012. Effect of feeding different levels of protein on growth performance, feed utilization and digestive enzyme of grey mullet (Mugil Cephalus L). Animal Nutrition and Feed Technology, 12: 179-186.

A growth trial was conducted to evaluate the effect of dietary protein level on growth performance, feed utilization and digestive enzyme activity of *Mugil cephalus*. Four isocaloric (4 kcal/g) experimental diets were formulated using locally available feed ingredients to contain different protein levels viz., 20, 25, 30 and 35% on DM basis and fed to triplicate groups (P20, P25, P30 and P35) of 25 grey mullet fry (average wt. ranging from 0.76 to 0.84 g), stocked in fibre glass tanks containing filtered brackish water and fed @ 10% BW twice daily. After 125 days of experiment, total weight gain in P20, P25, P30 and P35 were 2.82 ± 0.31, 3.10 ± 0.32, 3.81 ± 0.23 and 3.70 ± 0.16 (g) with corresponding feed conversion ratio of 7.69 ± 0.65, 6.36 ± 0.90, 4.86 ± 0.53 and 6.01 ± 0.25, respectively. The best growth performance and lowest feed conversion ratio ($P < 0.06$) in *Mugil cephalus* was obtained in group fed with 30% protein diet. Protein and lipid digestibility increased ($P < 0.01$) with increase in dietary protein level. Gut protease and cellulase activity were also significantly increased ($P < 0.05$ and $P < 0.01$) with dietary protein level up to 30%. Therefore, it can be concluded that diet containing 30 % protein is optimum for growth and digestibility of Mugil *cephalus* fry at 4 kcal/g energy level.

Key words: *Mugil cephalus*, Protein requirement, Growth, FCR.

INTRODUCTION

Grey mullets (*Mugil cephalus*) are widely distributed in seas and estuaries of tropics and subtropics. Among mullets *Mugil cephalus* is one of the commercially important brackishwater finfish species which is abundantly available in India (Biswas *et al.*, 2012; Jana *et al.*, 2004). It has very high consumer preference and fetch very good price in local market. Mullets have been increasingly used as a most suitable group for large scale fish farming in monoculture and polyculture. It plays a vital role as an efficient

*Corresponding author: Dr. Debasis De, E-mail: debasiskrc@yahoo.com

bioremediator in aquaculture (Lupatsch *et al.*, 2003) and improves sediment quality in culture pond due to its benthic feeding behavior. Culture practice of this species is not standardized due to uncertain seed availability and also scant information regarding their nutrient requirement and feed utilization. However, only a few studies on the formulation of suitable artificial diets have been reported (Papoutsoglou and Alexis, 1986). Nutritional quality of feed is critical for growth and survival of animals. Development of nutritionally balanced and cost-effective feeds is dependent on information on the nutritional requirements and feed utilization of the species. In nutritional studies, determination of their dietary protein requirement is generally considered as a primary step to formulate well balanced and low cost diets (Papoutsologlou and Alexis, 1986). Fishes require more proteins in their diets than warm-blooded animals. Excess dietary proteins are converted to energy source by some fishes. Proteins are not only the major constituent of animal's bodies, but also function as enzymes and hormones. Therefore, a continuous supply of proteins with balanced amino acids is required for maintenance and growth of target animal. Protein deficiency also causes cataracts and scoliosis in fishes, accompanied with a decline in the level of brain neurotransmitter, serotonin. On the other hand, if excessive proteins are provided, some of them are metabolized as energy; thus the most expensive dietary component will not be effectively used (Lee, 2004). Therefore, the present study was conducted to determine optimum protein requirement for maximum growth, for *Mugil cephalus* with a view to develop balanced and cost effective feed.

MATERIALS AND METHODS

Diet preparation and experimental protocol

For determining dietary protein requirement , four isoenergic (4 kcal/g) feeds with four different protein levels, 20, 25, 30 and 35%, were formulated and prepared as pellets (~ 1 mm) using locally available feed ingredients (Table 1). Chromic oxide was added as an inert marker @ 0.5 % in each feed to determine the digestibility.

Experimental animals

The fry of grey mullets, collected from Sunderban areas were acclimated to the laboratory condition for two weeks prior to the experiment. Juveniles of *M. cephalus* were divided randomly into four groups viz. P20, P25, P30 and P35 with three replicates in each. Twenty five fishes of average weight 0.57 ± 0.02g were stocked in separate FRP tanks containing 400 liters filtered brackish water and were kept in static indoor condition. Fishes of four groups P20, P25, P30 and P35 were fed with feed containing 20, 25, 30 and 35 per cent protein, respectively. A weighed quantity (10 % of BW) of diet was offered at 7 AM and 4 PM daily. Routine examination of reared fishes was done regularly for health assessment and fish sampling was done at fortnightly interval. Experiment was continued for 125 days.

Leftover feed was siphoned out and 50% of water of rearing tank was changed daily. Temperature, dissolved oxygen, pH, salinity and alkalinity of the water were measured (APHA, 1989) at weekly interval to assess the water quality in experimental

tanks. Leftover feed was recovered, dried and subtracted from the feed offered for computing the feed consumed. Faeces were collected through siphoning with plastic pipe two hours after each feeding continuously for 20 days, washed gently with distilled water, dried in the oven at 60°C for 8h and collected for digestibility studies.

At the end of the experiment ten experimental fishes from each tank were sacrificed. After evisceration, the whole intestine was homogenized with five times (w/v) of ice cold sterile physiological saline (0.9% NaCl in PBS buffer, pH 7.2). Homogenate was centrifuged at 10,000 rpm for 1h at 4°C and the supernatant was collected and used for enzyme assay.

Analysis of feed and faeces

Chemical analysis of feed and faecal sample was done following AOAC (1995) method and chromium oxide content of faecal matter was estimated by wet digestion method of Furukawa and Tsukahara (1966).

Enzyme assay

Cellulase activity was assayed (Denison and Koehn, 1977) using 1% CMC in citrate buffer (0.1 M, pH 6.75) as substrate. One specific cellulase activity unit (U) is defined as the amount of enzyme per mg of protein that released 1 μg D-glucose per minute. Amylase activity was measured (Bernfield, 1955) using 1% soluble starch in phosphate buffer (0.02 M; pH 6.9 containing 0.0067 M NaCl) as substrate. One specific amylase activity unit (U) is defined as the amount of enzyme per mg of protein that released 1 μg maltose per minute. Protease activity was assayed using 0.5% caseinase (Walter 1984) in Tris HCl buffer (0.02 M, pH 7.0) as substrate. One specific protease activity unit (U) is defined as the amount of enzyme per mg of protein that released 1 μg tyrosine per minute. Estimation of protein content was done spectrophotometrically (Lowry *et al.*, 1951) using 0.2 mg/ ml Bovine serum albumen (BSA) as standard.

Calculation and statistical analysis

The different parameters for evaluation of diets viz. digestibility coefficient, feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER) were calculated adopting standard procedures. At the end of the experiment fishes were analyzed for body composition. All the experimental data were subjected to one-way analysis of variance (ANOVA) to test the significance among the treatments (Snedecor and Cochran 1973). If group means were found significantly different they were compared using Duncan's multiple range test. All the parameters described under this experiment were analyzed using the software SPSS 17.0 (SPSS, IL USA).

RESULTS AND DISCUSSION

In the present study, temperature, dissolved oxygen, pH, salinity and alkalinity of the water ranged between 31.5-33.5 °C, 5.2-5.8 ppm, 7.54-7.69, 3.0-6.5 ppt and 118-124 ppm, respectively.

The proximate composition of experimental diets is presented in Table 1. In the present study, when fishes of P20, P25, P30 and P35 were given feed containing 20, 25, 30 and 35 % protein, respectively, total weight gain was highest in fishes fed 30 % protein (P30) and was significantly ($P < 0.06$) different from that of P20 (Table 2). FCR was lowest in P30 and was significantly ($P < 0.06$) different from that of P20 but similar to P25 and P35. PER and SGR was similar among the groups. The observation of the study revealed that the mean of total weight gain and SGR increased as the dietary protein level increased from 20 to 30% crude protein, and decrease at higher protein levels. The result depicted in the present study is practically the same as reported earlier in grass carp (Dabrowski, 1977), eel (Nose and Arai, 1972) and tilapia (Jauncey and Rose, 1982) and conforms with the general pattern observed for high quality proteins. But the results of present study are different from the study of Paul *et al*. (2009) who observed higher growth of *labeo bata* fry with continuous increase in protein in their diet. The weight gain of juvenile ivory shell with increasing dietary protein level from

Table 1. Ingredients and proximate composition of experimental feed with different level of protein

Particulars	Experimental diets			
	P20	P25	P30	P35
Ingredient composition (%)				
Wheat flour	36	16.5	18	10
Rice bran	23	25	10	10
MOC	22	30	30	19
Fish meal	10.5	20	33.5	52.5
Shrimp meal	5	5	5	5
Cr_2O_3	0.5	0.5	0.5	0.5
Mineral and vitamin mix.*	1.5	1.5	1.5	1.5
Guar gum	1.5	1.5	1.5	1.5
Proximate composition				
DM	92.56	96.75	96.98	98.53
OM	88.44	86.04	83.42	80.43
CP	20.11	25.29	30.30	35.19
EE	2.81	4.67	3.68	6.89
CF	4.25	4.33	4.68	4.29
Ash	11.56	13.96	16.58	19.57
AIA	5.74	6.49	6.00	8.57
NFE	61.27	51.75	44.76	34.06

*Mineral and vitamin mix. (per g): Vitamin A 2000 I.U., Vitamin D_3 400 I.U., Vitamin B_2 0.8 mg, Vitamin E 0.3 U, Vitamin K 0.4 mg, Calcium pantothenate 1mg, Nicotinamide 4 mg, Choline chloride 60 mg, Vitamin B_{12} 2.4 mg, Ca 0.3 g, Mn 11 mg, I 0.4 mg, Fe 3 mg, Zn 6 mg, Cu 0.8 mg, Co 0.18 mg.

Table 2. Performance of *Mugil cephalus* fed with diet of different levels of protein

Parameter	Dietary group				P-value
	P20	P25	P30	P35	
Initial BW (g)	0.57±0.02	0.54±0.03	0.55±0.05	0.64±0.02	0.247
Final BW (g)	3.39^{a}±0.29	3.58^{ab}±0.26	4.36^{b}±0.24	4.34^{b}±0.17	0.043
Total BW gain (g)	2.82^{a}±0.31	3.10^{ab}±0.32	3.81^{b}±0.23	3.70^{b}±0.16	0.056
Av. daily gain (mg)	22.56±2.44	24.80±2.55	30.48±1.86	29.60±1.24	0.080
FCR	7.69^{b}±0.65	6.36^{ab}±0.90	4.86^{a}±0.53	6.01^{ab}±0.25	0.059
PER	0.66±0.06	0.64±0.08	0.70±0.08	0.48±0.02	0.132
SGR (%)	1.42±0.10	1.52±0.11	1.66±0.08	1.54±0.03	0.321

[abc]Values bearing different superscripts in a row differ significantly.

27-43% was also reported by Zhou *et al*. (2007). Low dietary protein level produced higher FCRs, possibly due to intake of inadequate nutrient levels to promote growth (Zhou *et al.,* 2007; Shyong *et al.*, 1998; Ward *et al.,* 2003). PER increased with increased level of protein (20 to 30 %) and it started declining with further increase of dietary protein level, which was contradictory to findings of Zhou *et al*. (2007), Jauncey (1982) in *O. mossambicus*, Siddiqui *et al*. (1988) and Eid *et al*. (2003) in *O. niloticus* and other fish species who found significantly decreased PER with increasing level of protein up to 44% level.

Digestibility of CP and EE (Table 3) were similar among P25, P30 and P35 but significantly ($P<0.01$) higher than those of P20. No significant differences were observed in DM, OM, CF and NFE digestibility among the groups. Protein digestibility was increased with increased level of protein (20 to 30 %) and there after it tended to decrease. A positive correlation ($r=0.83$, $P<0.01$) was found between the digestibility of fat and level of dietary protein. Fat digestibility increased with the increasing level of protein. Crude fibre digestibility increased as the level of protein in diet increased but the difference was not significant.

Digestive enzyme assay revealed that protease activity was significantly ($P<0.05$) higher in P30 fed 30 % protein as compared to that of other groups (Table 4). Cellulase activity was also increased with the increasing level of dietary protein and it was maximum ($P<0.01$) in animal fed 30 % protein. Amylase activity did not differ due to difference in dietary protein level. Protease activity in the present study was increased with the increased level of dietary protein from 20 to 30 % but further increase of protein level reduced the protease activity. A positive correlation ($r=0.80$, $P<0.01$) was found between protein degrading enzyme activity and protein digestibility. This indicated that up to 30 % dietary protein level protein was optimally utilized and excess level of protein may

Table 3. Nutrient digestibility in *M. cephalus* fed different levels of protein in diet

Parameter	Dietary group			
	P20	P25	P30	P35
DM	76.51±0.91	78.69±0.26	80.51±1.62	77.67±0.39
OM	80.97±0.84	82.72±0.32	84.19±1.36	82.28±0.26
CP	82.86^{a}±0.28	85.40^{b}±0.25	86.78^{b}±0.89	85.92^{b}±0.10
EE	84.49^{a}±0.88	86.86^{b}±0.52	87.48^{b}±0.34	88.24^{b}±0.26
CF	54.21±1.68	66.99±10.09	67.81±13.18	45.85±3.03
NFE	83.88±1.37	84.27±0.28	84.88±0.55	83.16±0.18

^{ab}Values bearing different superscript in a row differ significantly ($P<0.01$).

Table 4. Effect of dietary protein level on the activity of digestive enzyme of *M. cephalus*

Group	Protease (U/mg protein)*	Amylase (U/mg protein)	Cellulase (U/mg protein)**
P20	5.21^{a}±0.06	31.30±0.21	43.79^{a}±0.19
P25	5.66^{ab}±0.05	31.85±0.99	45.45^{b}±0.24
P30	6.12^{b}±0.28	32.87±1.55	48.11^{c}±0.89
P35	5.62^{a}±0.06	31.57±0.58	45.14^{ab}±0.16

^{abc}Values bearing different superscript in a column differ significantly (*$P<0.05$; ** $P<0.01$).

be utilized for energy supply to the fishes which may not be recommended. Cellulase activity also showed the similar trend as found for protease activity which revealed that up to 30 % dietary protein level nutrient utilization and digestibility was optimum.

Body composition (Table 5) analysis revealed that protein was significantly ($P<0.01$) higher in P25, P30 and P35 than that of P20, but no difference was observed

Table 5. Body composition (% DM basis) of *M. cephalus* fed different level of protein

Components	Dietary group			
	P20	P25	P30	P35
OM	83.47±0.38	82.76±0.85	83.43±0.21	82.01±0.48
CP	49.67^{a}±0.19	51.27^{b}±0.16	51.34^{b}±0.18	52.03^{b}±0.47
EE	27.04^{c}±1.13	24.68^{b}±0.37	26.56^{bc}±0.52	21.54^{a}±0.09
Ash	16.53±0.38	17.24±0.85	17.57±1.21	17.99±0.48
AIA	0.84^{c}±0.12	0.38^{ab}±0.01	0.58^{b}±0.01	0.31^{a}±0.05
NFE	6.76±1.18	6.81±0.71	4.53±1.55	8.44±0.97

^{abc}Values bearing different superscript in a row differ significantly ($P<0.01$).

among P25, P30 and P35. Body protein content of fish fed 25, 30 and 35 % protein diets were similar as excess dietary protein might have been catabolized for energy (Wilson, 1989). Body fat did not show any definite trend with the increased level of protein in diet and it was significantly ($P < 0.01$) higher in P20 and P30 as compared to that of P25 and P35. Muscle protein content of the fish was significantly increased with increasing level of dietary protein and fish fed high-protein diets tended to have lower muscle lipid content which was supported with the similar results in common carp (Zitter *et al*.,1984), young grey mullet (Papoutsologlou and Alexis, 1986) and Nile tilapia (Eid *et al*., 2003) . In case of ash content, it was unaffected by different dietary protein levels, as was reported with other fish species (Jauncey, 1982; Siddiqui *et al.,* 1988 and Eid *et al*., 2003). There was an inverse relationship between the body moister and lipid content, in agreement with Jauncey (1982) and Eid *et al*. (2003).

Based on the results, it can be concluded from the present study that *Mugil cephalus* require 30% protein for its optimum growth and digestibility.

ACKNOWLEDGEMENT

The authors express their thanks to Dr. A.G. Ponniah, Director, CIBA, Chennai for providing necessary facilities to conduct the experiments.

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