Effects of dietary protein and energy levels on growth performance, feed utilization and body composition of juvenile shirbot *Barbus grypus* (Heckle, 1843)

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Abstract

Shirbot (*Barbus grypus*) as a species with high potential for aquaculture development recently propagated artificially in South Iran Aquaculture Research Center to extend the species diversity in polyculture system. To provide an effective economic diet for this species 9 experimental diets containing three crude protein levels (250, 300, and 350 g kg $^{-1}$) and three metabolizable energy levels (10.46, 12.55 and 14.64 MJ kg $^{-1}$) were fed to triplicate groups of shirbot juvenile fish (initial body weight of 29.68± 0.19). Three aerated tanks were randomly assigned to each treatment, stocked with 15 juvenile fish and reared for a 60-day period. The preferential diet, which was diet 4, (300 g kg $^{-1}$ CP and 10.46 MJ kg $^{-1}$ ME) exhibited the best growth and feed utilization performances. Fishes fed diet 4 showed higher weight gain, feed efficiency ratio and survival rate with a significant difference ($P<0.05$) for WG and FER than other diets except diet 2. It was revealed that increase of CP level in the diet leads to an increase of crude lipid and fiber in the body composition of the fish, but adverse results were obtained when diet ME was increased. Apparent Net Protein Utilization value was increased when the diet protein and energy level were low but the difference was not significant ($P>0.05$). It was found that varying levels of CP and DE in the diets did not significantly affect the body composition of the fish ($P>0.05$) except for the CF. Comparison between varying levels of dietary protein and energy on the growth, feed utilization and body composition of *Barbus grypus* indicated that 250 to 300 g kg -1 CP and 10.46 MJ kg -1 ME could be the preferential dietary levels for this species in the juvenile stage.

Keywords: *Barbus grypus*, Juvenile , Dietary protein level Dietary energy level

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Introduction

Shirbot (*Barbus grypus* Heckel, 1843) is mainly spread in Euphrate Basins (Coad, 1979). High growth performance, broad feeding regime as an omnivorous species and high handling resistance as well as its high domestic demand (Ghafleh Marammazi, 2000) recently led to its artificial propagation in South Iran Aquaculture Research Center (SIARC) for the first time in the world (Moazedi et al., unpublished). Providing a well formulated diet containing well-balanced protein and energy for this species is firmly required to economize its rearing enterprise in commercial pond culture. Protein is the dietary nutrient whose requirement is prioritized in nutritional studies, either because it represents the highest fish feed cost, or by greatly affecting growth (Meyer and Fracalossi, 2004). Studies on varying levels of both dietary protein and energy concentration have demonstrated the fish capability to spare protein when other energy sources such as carbohydrates and lipid are added to the diet (McGoogan and Gatlin, 2000).

Dietary energy regulates the feed intake and so greatly affects the growth, protein efficiency ratio, body lipid accumulation, financial profit and the quality of pond water (Lovell, 1998). If the dietary P/E ratio is unbalanced and the non-protein energy is inadequate, dietary protein may be catabolized and used as an energy source to meet the maintenance before growth (NRC, 1983; Cowey and Sargant, 1979).

To date, estimated findings of the studies indicated that protein requirements of the fish species are to be in the range of 400 to 550 g kg $^{-1}$ for the carnivorous fish (NRC, 1983), and 250 to 350 g kg $^{-1}$ for most herbivorous and omnivorous fishes. Accordingly carnivorous species may require CP between 400 and 500 g kg -1 (NRC, 1993; Gatlin, 2001). Shiau and Lan (1996) reported a decrease in grouper fingerling crude protein (CP) requirement from 500 to 400 g kg $^{-1}$ when dietary energy content increased from 12.61 to 14.23 MJ kg $^{-1}$.

Energy and nutrient requirements are affected by several factors and they may vary in different stages of the life cycle of the animal. Several authors have described optimal dietary P/E ratios in some commercial rearing species such as rainbow trout, *Oncorhynchus mykiss* (Kim and Kaushik, 1992; Lanari et al., 1995), African catfish, *Clarias gariepinus* (Henken et al., 1986; Ali and Juancy, 2005), Atlantic salmon, *Salmo salar* (Hilles-tad and Johnsen, 1994), common carp, *Cyprinus carpio* (Watanabe et al., 1987), Asian sea bass, *Lates calcarifer* (Nankervis and Southgate, 2006), gilthead sea bream, *Sparus aurata* (Vergara et al., 1996 b), jundia, *Rhamdia quelen* (Meyer and Fracalossi, 2004) , Nile tilapia, *Oreochromis niloticus* L (Liti et al., 2006) and African Cichlids, *Pseudotropheus socolofi* and *Haplochromis ahli* (Royes and Murie, 2006).

The objective of the present study was to evaluate the effects of varying levels of dietary protein and energy on the growth, feed utilization and body composition of shirbot juveniles and to specify the optimum dietary level for this fish.

Materials and methods

Diet preparation

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With the consideration of three crude protein (CP) levels: P1 (250 g kg $^{-1}$) P2 (300 g kg $^{-1}$) and P3 (350 g kg $^{-1}$), and three metabolizeable energy (ME) levels: E1 (10.46 MJ kg $^{-1}$; MJ=Mega Joule) , E2 (12.55 MJkg $^{-1}$) and E3 (14.64 MJ kg $^{-1}$ diet), 9 experimental diets (D) as (D1: P1E1, D2: P1E2, D3: P1E3, D4: P2E1, D5: P2E2, D6: P2E3, D7: P3E1, D8: P3E2, D9: P3E3) were formulated using Lindo Software (Scharge, 1991). All the ingredients used in this study were purchased from the local market (Table 1). Available energy of the test diets was calculated using physiological fuel value of 16.7, 16.7 and 37.7KJ g^{-1} for protein, carbohydrates and lipid, respectively (Du et al., 2005; Meyer and Fracalossi, 2004). An appropriate amount of each ingredient was weighed and mixed for 20 minutes. Vitamin premix was first diluted in soy oil, then added to the ingredients mixture and mixed again for another 20 minutes. Lukewarm tap water with the ratio around 300g kg $^{-1}$ of the ingredients mixture was gradually added along with the mixing, so that stiff dough was produced. Dough was changed to pellet feed using mincing machine with the die of 2mm diameter. Wet pellets were dried at room temperature for around 24 hours. Use of provided diets started right after preparation and they were kept in room temperature during experiment implementation.

Preparation of Fish

Artificially propagated shirbot juveniles were provided by the SIARC hatchery center. After one week of acclimatization in a 4000L tank, they were weighed (29.68 ± 0.19 g) and 15 individuals were released in each 300L round polyethylene tank. 27 similar tanks were randomly assigned to 9 treatments in 3 replicates. Daily feed ration for the fish was determined based on it's biomass and balanced to the satiation point of fishes. Daily feed ration was divided in to three parts and given to the fish at 08:00, 14:00 and 20:00 h. Remained feed pellets in each tank were counted one hour after feeding, in order to have the accurate amount of consumed feed.

Growth Performance

Fish weight (W) and their number were recorded at every 15 days interval during the 60 days rearing period, to prevent injury of fish during the biometric operation, they were anaesthetized with 0.3 g l^{-1} of ethylonglycol monophenol (EGMP). At the end of the experiment, fish weight and number and the amount of the feed consumed in each tank were recorded to calculate the following growth indices.

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Table 1: Composition of experimental diets (g kg $^{-1}$ dry weight)

ingredients	Experimental diets								
	D1 (P1E1)	D2 (P1E2)	D3 (P1E3)	D4 (P2E1)	D5 (P2E2)	D6 (P2E3)	D7 (P3E1)	D8 (P3E2)	D9 (P3E3)
Fish meal[1]	170	190	262.5	370	383.5	387.5	512.5	512.5	512.5
Soybean meal	319.9	363.6	246.5	225.2	231.4	255.2	216.3	245.8	269.4
Corn	160	170	120	140	120	33.4	80	81.1	-
Barley	140	90.4	41.8	57.3	23.2	-	70	-	-
Wheat	30	100	40	100	50	50	50	50	50
Rice bran	10	50	20	30	30	30	30	30	2.4
Wheat bran	146	-	100	70	70	70	32.6	-	-
Soy oil	20	70	165.1	3.4	83.8	169.9	5.6	77.6	162.7
Vit. Premix [2]	1.5	0.7	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Min. premix [3]	2.5	2.5	2.5	2.5	2.5	2.5	1.5	1.5	0.15
Proximate composition									
Crude protein	245.5	247.5	242.4	291.1	293.2	297.8	350.3	344.4	347.5
Crude lipid	215.5	214.7	238.2	174.3	174.1	231	222.6	281.6	281.3
Carbohydrate (NFE) [4]	369.3	358.8	345.3	334.8	357.0	298.1	262.9	196.2	192.4
Metabolizable energy (MJ kg $^{-1}$)	10.39	12.45	14.51	10.42	12.37	14.65	10.45	12.41	14.57
Moisture	76	77.3	71.8	68.2	75.7	78.3	83.1	85.6	88.2
Ash	47.3	49.3	48.3	47.8	46.7	43.4	48.6	50.1	48.5
Crude fiber	36.4	52.4	54	83.8	53.3	51.4	32.5	42.1	41.1

[1] Produced from kilka (clupeid from the Caspian Sea).

[2] Vit. premix contents (mg or IU per gram premix): A, 1600 IU; D3, 400 IU; E, 40; K3, 2; B1, 6; B2, 8; B5, 40; B6, 4; B9, 2; B12, 8; C, 60; H2, 0.24; Inositol, 20; BHT,20; Carrier, up to 1 g)

[3] Min. premix contained (mg per gram premix): Iron, Zinc, 12.5; Selenium, 2; Cobalt, 480; Copper, 4.2; Magnesium, 15.8; Iodine, 1; Choline chloride, 12; Carrier, up to 1g.

[4]Nitrogen free extract

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Weight gain (WG) = final weight–initial weight; Specific growth rate (SGR) = [(ln final weight-ln initial weight×100)/days of rearing]; Survival rate (SR) = final number of fish individuals ×100/initial number; Food conversion ratio(FCR) = food consumed(g as fed)/weight gain (g wet); Protein efficiency ratio (PER) = weight gained(g wet)/protein consumed(g); Apparent net protein utilization [(ANPU) =100×(final body protein- initial body protein)/protein consumed] (Meyer and Fracalossi, 2004).

Sampling and Analysis

At the beginning of the experiment, 30 fishes were randomly captured after 24 hours fasting and frozen for initial body analysis. At end of the experiment, three individuals were separated from each replicate after 24 hours fasting and frozen for final body composition analysis. Samples of food ingredients and the carcass were analyzed based on AOAC (1990) methods. The moisture was measured by oven drying at 105 ◦C for 24 hours. Protein, lipid, fiber and ash of formulated diets and fish carcass were analyzed by the Kjeldahl method with Tecator kjeltec (1030-Auto-Analyzer), soxhlet extraction with tecator soxtec, fibertec system and combustion in muffle furnace at 550 ◦C for16 hours, respectively.

Statistical Analysis

Data, which were expressed as mean ± SD, were analyzed using SPSS software (ver. 14). Comparison between means was performed by two way ANOVA method and Tukey test; a confidential level of 95% was considered in all tests.

Results

Growth performance and feed utilization indices of shirbot juveniles exposed to experimental diets containing varying levels of dietary protein and digestible energy are summarized in Tables 2, 3 and 4. Fish fed D4 exhibited the highest growth performance with a significant difference ($p<0.05$) from those fed diets D6 and D9 in terms of WG and SGR parameters. Furthermore, the best FCR value was observed for D4, but the highest value was recorded for diet D2**.** Fish fed diet D2 exhibited best value in terms of PER with significant difference ($p<0.05$) compared to those fed diets D6, D7, D8 and D9. Higher value of ANPU was recorded for the fish fed diets D3, D1, D4 and D2, respectively, while the lowest value was recorded for D9, although the difference is not significant ($p>0.05$). Varying dietary protein and energy did not effectively influence survival rate (SR) of shirbot juveniles and the value of this parameter was efficiently high for all the diets. When dietary protein level elevated from P1 to P2, FCR, WG and SGR were improved, but they exhibited a reversed trend when the dietary protein elevated to P3. Elevation of dietary protein level from P1 to P3 resulted in a significant decrease ($p<0.05$) in PER value. A similar trend was also observed for ANPU (Table 2), but without a significant difference ($p>0.05$).

Elevation of dietary energy level from E1 (10.46 MJ kg $^{-1}$diet) to E3 (14.64 MJ kg $^{-1}$diet) significantly decreased ($P<0.05$ WG and SGR. A similar trend was also observed for ANPU, however the difference was not significant ($p>0.05$).

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The best FCR value was recorded for fish fed diets containing E1 followed by E3, and the highest was for those fed E2 (12.55 MJ kg $^{-1}$diet) dietary energy level. An inverse trend was observed for PER (Table3), although the difference was not significant ($p>0.05$) for both parameters (FCR and PER).

Dietary protein and energy variation did not significantly affect ($p>0.05$) the chemical composition of Shirbot, except for fiber; however, the highest body protein content was recorded in fish fed diet D5, followed by those fed diets D3, D8 and D4 respectively. Fish fed diet D7 showed the highest body fat content, followed by those fed diet D9. The lowest fat was observed in fish fed diet D3 (Table 5). The content of carcass ash was not significantly influenced by dietary protein or energy levels ($p>0.05$), however higher values were observed in fish fed diet D8 and diet D3 as well as those fed diets with P2 protein level (Table 6).

Body fiber of Shirbot was effectively influenced by dietary protein and energy levels and their interaction. Fish fed diet D4 and diets containing E1 energy level exhibited significantly higher body fiber ($p<0.05$) than those fed diet D1 and diets containing E3 energy level.

Discussion

Results of the present study demonstrated that growth performance and food efficiency of Shirbot *B.grypus* improved in diets containing P1 (250 g kg^{-1}) and P2 (300 g kg^{-1}) levels of dietary crude protein and E1 (10.46 MJ kg^{-1} diet) level of digestible energy. Also it was revealed that tested diets did not perform well in terms of ANPU and PER when dietary protein increased. Similar results were observed in terms of ANPU and WG when dietary energy increased. Performance fluctuation was observed in terms of other indices when dietary protein or energy increased.

To date, there is no documented data which explains the dietary requirements of Shirbot or other Barbus species. Therefore, the results of the present study could be compared only with the investigations carried out on the feed requirements of some omnivorous or herbivorous species belonging to cyprinid or other families. Results of the present study are likely to be in great correspondence with the results obtained on omnivorous fishes. Winfree and Stickney (1981) reported that *Tilapia aurata* fed a diet containing 34% crude protein and 3200 Kcal kg^{-1} digestible energy and P/E of 108 mg CP kg^{-1} DE resulted in better growth and FCR than those fed diets with 56% CP and 4600 Kcal kg^{-1} digestible energy. Relatively similar results are explained for fry and juvenile stages of *Oreochromis niloticus* tilapia by Siddiqui et al. (1988). They suggested 30% dietary protein for juveniles and 40% for the fry stage of this species. Findings of Jauncy (1982) on tilapia correspond greatly with the mentioned reports. Also catfish of *Clarias batrachus* showed best performance in terms of growth rate, PER and FCR with 30% dietary protein. According to the findings of Seenappa and Devaraj (1995) best growth performance and body composition of catla, *Catla catla* was observed with a diet containing 30% to 35% dietary protein. Almost similar results were obtained by Murthy and Naik (2000) for this species.

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Table 2: Growth performance and feed utilization indices of shirbot juveniles fed different dietary protein and energy levels

	Experimental diets								
	D1 (P1E1)	D2 (P1E2)	D3 (P1E3)	D4 (P2E1)	D5 (P2E2)	D6 (P2E3)	D7 (P3E1)	D8 (P3E2)	D9 (P3E3)
Growth performance									
WG(g)	253.3±41.5 ^{ab}	266.0±31.2^{ab}	245.0±50.3 ^{ab}	329.3±20.6 ^{a}	260.7±27.0 ^{ab}	230.7±18.7^{b}	291.0±20.6 ^{ab}	282.7±41.9 ^{ab}	224.7±30.2 ^{b}
SGR	0.8 ± 0.1 ^{ab}	0.8 ± 0.1 ^{ab}	0.7 ±0.1 ^{ab}	0.9 ± 0.1 ^{a}	0.8± 0.1 ^{ab}	0.7± 0.1 ^{ab}	0.8±0.1 ^{ab}	0.8±0.1 ^{ab}	0.7±0.07 ^{b}
SR(%)	97.8± 3.6	100	100	100	100	100	100	100	100
Feed utilization									
FCR	3.4±0.8	4.0±2.0	3.8±1.6	2.6±0.2	3.7±0.5	3.3±0.6	3.5±2.1	3.6±0.4	3.0±0.2
PER	1.7±0.5 ^{ab}	2.4±0.9 ^{a}	1.6±0.2 ^{ab}	1.5±0.1 ^{ab}	1.4±0.3 ^{ab}	1.3±0.1 ^{b}	1.3±0.1 ^{b}	1.2±0.2 ^{b}	1.1±0.1 ^{b}
ANPU	75±61	73±64	79±23	74±9	66±26	58±17	51±14	58±10	45±13

Figures in the same row with similar superscript are not significantly different (p>0.05)

Table 3: Growth performance and feed utilization indices of shirbot juveniles fed different dietary protein levels

Protein level	FCR	PER	WG (g)	SGR	ANPU
P1	3.7 ±0.4	1.9 ± 0.1 ^{a}	257.8 ±11.0	0.8 ± 0.0	8±1
P2	3.2 ±0.4	1.4 ± 0.1 ^{b}	273.6±11.0	0.8 ± 0.0	7±1
P3	3.4±0.4	1.2 ± 0.1 ^{b}	266.1 ±11.0	0.8 ± 0.0	5±1

Figures in the same column with similar superscript are not significantly different (p>0.05)

Table 4: Growth performance and feed utilization indices of shirbot juveniles fed different dietary energy levels

Energy Level	FCR	PER	WG (g)	SGR	ANPU
E1	3.1± 0.4	1.54 ± 0.1	294.2±11.0^{a}	0.8± 0.0 ^{a}	67±1
E2	3.7 ± 0.4	1.7 ± 0.1	269.8±11.0^{ab}	0.8 ± 0.0 ^{a}	64±1
E3	3.4 ± 0.4	1.3± 0.1	233.4±11.0^{b}	0.7 ± 0.0 ^{b}	61±1

Figures in the same column with similar superscript are not significantly different (p>0.05)

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Table 5: Body composition of shirbot juveniles at the beginning[1] and at the end of the experiment

	Experimental diets								
	D1	D2	D3	D4	D5	D6	D7	D8	D9
***Final body composition* (%)**									
Crude protein	53.1±13.6	51.6±6.4	57.7±7.6	54.2±4.3	58.4±6.3	53.4±3.9	52.2±3.7	57.2±2.6	50.3±3.7
Crude lipid (EE)	19.3±5.4	17.9±5.7	17.6±12.9	26.7±11.5	18.4±14.2	18.2±12.9	30.2±8.9	19.6±9.9	30.0±7.5
Moisture	2.6±.2	3.5±1.9	1.7±.5	2.0±.8	1.7±.4	1.6±.4	1.8±.4	2.1±.8	1.8±.1
Ash	7.0±1.7	6.6±1.3	7.9±0.8	7.5±0.3	7.5±0.3	7.7±0.13	7.7±0.2	7.9±0.3	6.71±0.6
Crude fiber	3.2±.1.6^{abc}	4.2±.1.4^{abc}	2.1±0.9^{c}	6.1±0.9 ^{a}	2.85±.1^{bc}	2.8±1.9^{bc}	4.03±.1.8^{abc}	5.4±2.3^{ab}	2.6±.1.9^{bc}

Figures in the same row with similar superscript are not significantly different (p>0.05)

[1]At the begining of the experiment 10 fishes were randomly seperated from those used for rearing and analysed after drying accordingly. Body composition at the begining was as follow: CP=52.1%, EE= 10.8%, CF= 1.4%, Moisture=3.6% and Ash= 4.5%

Table 6: Body composition of shirbot juveniles at the end of the experiment

Prot. level	Prot.Content (%)	Lipid Cotent (%)	Fiber Content (%)	Ash Content (%)
P1	54.2±2.6	18.3±3.4	3.2±0.5	7.1±0.3
P2	55.3±2.6	21.1±3.4	3.9±0.5	7.6±0.3
P3	53.2±2.6	26.6±3.4	4.0±0.5	7.4±0.3

Figures in same column with same superscript are not significantly different (p>0.05)

Table 7: Body composition of shirbot juveniles at the end of the experiment

Ener. Level	Prot.Content (%)	Lipid Cotent (%)	Fiber Cont.ent (%)	Ash Content (%)
E1	53.2±2.6	25.4±3.4	4.5±0.5 ^{a}	7.4±0.3
E2	55.7±2.6	18.6±3.4	4.2±0.5 ^{ab}	7.3±0.3
E3	53.8±2.6	21.9±3.4	2.5±0.5 ^{b}	7.4±0.3

Figures in the same column with similar superscripts are not significantly different (p>0.05)

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Many authors have reported that increase in dietary protein up to an optimum level has led to higher growth rates in the target species, beyond which not only it does not support the growth, but also may decrease it (Mohanty and Samantary 1996; Shiau and Lan, 1996; McGoogan and Gatlin, 1999; Gunasekara et al., 2000; Kim and Lall, 2001; Yang et al., 2002). Moreover, results of the present study indicated decline in growth and feed performance of Shirbot when dietary protein level exceeded 300 g kg $^{-1}$, and therefore the findings are in correspondence with those obtained from aforementioned studies.

Increase in dietary energy beyond the optimum level may spare dietary protein and lead to better economy profit for some species such as jundia, *Rhamdia quelen*, when its dietary energy increased from 13.39 to 15 MJ kg^{-1} (Meyer and Fracalossi, 2004). Several similar reports on protein sparing are available on Indian major carp, *Catla catla* (Seenappa and Devaraj, 1995), Asian sea bass, *Lates calcarifer* (Catacutan and Coloso, 1995), Atlantic salmon, *S. salar* (Hillstad and Johnsen, 1994), and rohu, *Labeo rohita* (Satpathy et al., 2003). Results of the present study did not indicate such events for Shirbot species.

Body composition of Shirbot was not evidently influenced by the dietary protein and energy interaction except for crude fiber, which was significantly ($P<0.05$) affected by energy levels and the interaction of dietary protein and energy. El-Sayed et al. (2008) found that body composition of *Oreochromis niloticus* tilapia brooders have not been influenced by the varying levels of dietary protein and energy except for crude protein. However results obtained on *Oreochromis niloticus* and *Xiphophorus hellari* showed non significant effects of dietary protein and lipid on the brooders body composition of these species (El-Sayed et al., 2000; Chang et al., 2004). Also when dietary energy increased in the present study, body crude lipid of this fish was not significantly increased as it has been reported on rohu, *Labeo rohita* (Afzalkhan et al., 2005), Murray cod, *Maccullochell peelii peelii* (De Silva et al., 2002) and gilthead sea bream, *Sparus aurata* (Company et al., 1999). Higher levels of dietary energy may need to be examined on shirbot for explaining this subject.

The estimated dietary protein and energy requirements of some omnivorous species found to be: 326- 373 g kg -1 CP for jundia (Meyer and Fracalossi, 2004); 280–320 g kg $^{-1}$CP for channel catfish (Robinson et al., 2000); 430 g kg $^{-1}$ Cp , 21.2 MJ kg $^{-1}$of GE for Aferican catfish; 300- 360 g kg $^{-1}$, for Nile tilapia (Shiau, 2002); 350 g kg $^{-1}$ CP (NRC,1993) and 300 -350 g kg $^{-1}$ Cp , 12.97 – 15 MJ kg $^{-1}$of DE (Webester and Lim, 2002) for common carp; 300 – 400 g kg $^{-1}$ CP, 7.62 MJ kg $^{-1}$ DE (Du et al., 2005) for grass carp; 350 g kg $^{-1}$ CP, 18.43 MJ kg-1 (4400 kcal kg $^{-1}$) DE (Satpathy et al., 2003) , 350 g kg $^{-1}$ CP and 15.7 MJ kg $^{-1}$ of DE (Afzalkhan et al ,2003) for rohu; 15.7 MJ

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kg $^{-1}$ DE and 350 g kg $^{-1}$ CP (Mishra and Samantary, 2004) for mrigal.

According to the findings of the present study and to those information obtained from other species, it could be concluded that protein and energy requirements of shirbot as an omnivorous species is placed in the range of 250-300 g kg $^{-1}$ protein and 10.46 MJ kg $^{-1}$ energy, which correspond well with the covering dietary protein and energy requirements of other omnivorous species. These levels of protein and energy might be good enough for juvenile stage but protein and energy requirements of other stages of this species should be described by further studies.

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