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Growth performance and body composition of kutum fingerlings, *Rutilus frisii kutum* (Kamenskii 1901), in response to dietary protein levels

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Abstract: A feeding trial was conducted to determine the optimal dietary crude protein requirement for *Rutilus frisii kutum* fingerlings. Five isoenergetic experimental diets were formulated to contain graded levels of protein (32%, 37%, 42%, 47%, and 52%). Triplicate groups of 80 kutum (average weight 0.5 g) were stocked in 250-L tanks and fed to apparent satiation 3 times a day for 8 weeks. The results indicate that the growth performance and feed utilization of kutum were significantly ($P < 0.05$) affected by dietary protein level. The second polynomial regression of weight gain against protein level yielded an estimated optimal dietary protein requirement of 41.6%. Weight gain, specific growth rate, and feed efficiency increased with increasing the dietary protein level from 32% to 42%, but decreased significantly ($P < 0.05$) with further increases in dietary protein. The protein efficiency ratio was inversely correlated with dietary protein level. Feed intake was not affected ($P > 0.05$) by dietary protein level. No significant difference was found in the whole-body protein, lipid, moisture, or ash contents of fish fed the different dietary protein levels. The results obtained from this study could be beneficial in order to formulate artificial feed for aquaculture of kutum fingerlings.

Key words: *Rutilus frisii kutum*, protein requirement, growth, fish diet, protein efficiency

Introduction

Kutum, *Rutilus frisii kutum* (Kamenskii, 1901; family Cyprinidae), locally known as mahi sefid, is an important commercial fish in Iran. This species is native to the Caspian Sea and distributed from the mouth of the Volga River up to the Astrabadskiy Bay. The main population of kutum is located at the southern coast of the Caspian Sea from the Atrak River to the Kura River (Caspian Environment; Razavi, 1997; Abdoli and Naderi, 2009). Kutum is a very popular fish for human consumption, especially in northern Iran, as this fish species comprises an important part of the local diet during the fishing season, from the beginning of October to mid-April. Kutum is a migratory anadromous fish that spawns on aquatic weeds, gravel, and sandy substrate in rivers and lagoons. The spawning season of the kutum in the rivers of the southern part of Caspian Sea begins in late March and ends in mid-May (Azari Takami, 1990). A sharp decline in the annual catch of kutum observed in the 1970s and early 1980s was likely due to declining natural populations, exacerbated by the destruction of natural spawning areas and other factors (Ghaninejad and Abdulmaleki, 2007), and, since then, the natural reproduction of kutum

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has been limited. As a result, the Iranian fisheries organization (Shilat) launched a restocking project in 1984. This project seems to have been successful in increasing kutum biomass in the Caspian Sea: the annual landing of kutum increased from 563 t in 1982 to 16,118 t in 2006 (Salehi, 2008).

To restock this valuable species, the Shilat annually produces and releases more than 200 million fingerlings into rivers, which carry them towards the Caspian Sea (Heyrati Paykan et al., 2007). In the restocking centers, larval rearing takes place in earthen ponds. Kutum larvae are grown in the ponds until a releasing weight of 1 g is attained.

The successful production of kutum in hatcheries means that a reliable supply of fingerlings is now available for commercially culturing this species. Success of aquaculture is affected by several factors. One important factor is the formulation of balanced diets for the fish that provide the required nutrients to support good growth and health (Salhi et al., 2004). Because protein plays a key role in the growth of fish, knowledge about the protein requirements of each species is essential for the formulation of species-specific diets. Inadequate protein in diets will result in a reduction or cessation of growth. On the other hand, if excessive dietary protein were used in diets, only a part of this protein would be used to synthesize new proteins for growth and repair, whereas the rest would be converted to meet the energy requirements of the fish (Willson, 2002; Salhi et al., 2004). The optimum use of protein for the promotion of growth is essential so as to make aquaculture commercially viable and also to prevent organic loading in the ecosystem, which ultimately leads to water pollution through a decrease in ammonia nitrogen excretion (Kaushik and Oliva-Teles, 1985).

Although several studies have been conducted to determine the nutritional requirements of kutum (Fallahi et al., 2004; Noverian et al., 2008; Noverian and Shabanipour, 2008), there is no accurate information specifically on their protein requirements. Therefore, the present study was designed to estimate the optimum dietary protein level and determine its effect on growth performance, feed utilization, and body composition of *R. frisii kutum* fingerlings.

Materials and methods

Experimental diets

Five isoenergetic experimental diets were formulated to contain graded protein levels (32%, 37%, 42%, 47%, and 52%, wet weight basis). Ingredients were obtained from a local feed manufacturer (khorak dame mazandran, Sari). To determine the energy content of the food, the amounts of protein, fat, and carbohydrate in each diet was determined. The gross energy content of each diet was then calculated based on 23.4, 39.2, and 17.2 MJ/kg for protein, lipid, and nitrogen-free extract, respectively (Kim and Lee, 2005). The composition and proximate analysis of the experimental diets are shown in Table 1. The experimental diets were prepared by mixing the dry ingredients in a Pars electric mixer; then oil and sufficient water was added to the dry mixture to form a soft dough (approximately 40% to 50% moisture). The mixed dough was extruded through an electric meat grinder and dried at 60 °C for 6 h in a drying oven. Diets were packed and stored at –20 °C until utilization.

Fish and feeding trial

Kutum fingerlings were obtained from the Shahid Rajaee restocking center located on the southern coast of the Caspian Sea, Sari, Iran. In this center, kutum were kept in earthen ponds and fed on natural live food and commercial food. Before starting the experiment, the fish were acclimated to the experimental conditions for 2 weeks. During acclimation, the fish were fed commercial carp feed (30% protein) twice daily. After acclimation, kutum fingerlings with an initial body weight of 0.5 ± 0.09 g were randomly distributed into 15 fiberglass tanks of 250 L capacity with 80 fish per tank. Each dietary treatment had 3 replicates. All of the tanks were covered with nets throughout the experiment, to prevent the fish from jumping out. Freshwater was supplied at a flow rate of 1.5 L/min to each tank. Water quality parameters were checked periodically; pH ranged between 7.8 and 8.0; water temperature ranged from 19.7 to 23.2 °C, and dissolved oxygen was never less than 7.2 mg/L. Fish were held under natural photoperiod conditions throughout the feeding trial.

Table 1. Formulation and proximate composition (wet weight) of the experimental diets.

Ingredient (%)	Dietary protein level (%)				
	32	37	42	47	52
Fish meal[a]	38	46	54	63	71
Wheat flour	37.8	34	30	20	15
Fish oil[b]	4.7	4.1	3.4	2.7	2
Soybean oil	3.8	3.8	3.85	3.9	3.9
Mineral mixture[c]	2	2	2	2	2
Vitamin mixture[d]	2	2	2	2	2
Cellulose[e]	11.7	8.1	4.75	6.4	4.1
Proximate composition (%)					
Moisture	10.24	10.48	9.68	8.9	8.16
Crude protein (Calculated)	32	37	42	47	52
Crude protein (Analyzed)	33.3	36.23	41.40	46.88	51.96
Lipid	11.66	12.33	11.93	13.16	12.69
Ash	8.07	8.78	9.48	10.56	12.85
NFE[f]	36.72	32.17	27.49	20.5	14.33
Gross energy (MJ/kg)[g]	18.68	18.84	19.09	19.65	19.60
P/E ratio[h]	17.82	19.22	21.68	23.85	26.50

[a]Pars kelika Co., Mirood, Iran

[b]Herring oil

[c]Vitamin premix contained the following vitamins (each kg^{-1} diet): vitamin A, 10,000 IU; vitamin D_3 2000 IU; vitamin E, 100 mg; vitamin K, 20 mg; vitamin B_1, 400 mg; vitamin B_2, 40 mg; vitamin B_6 20 mg; vitamin B_{12}, 0.04 mg; biotin, 0.2 mg; choline chloride, 1200 mg; folic acid, 10 mg; inositol, 200 mg; niacin, 200 mg; and pantothenic calcium, 100 mg.

[d]Contained (g kg^{-1} mix): $MgS0_4.2H_2O$, 127.5; KCl, 50.0; NaCl, 60.0; $CaHPO_4.2H_2O$, 727.8; $FeSO_4.7H_2O$, 25.0; $ZnSO_4.7H_2O$, 5.5; $CuSO.5H_2O$, 0.785; $MnSO_4.4H_2O$, 2.54; $CoSO_4.4H_2O$, 0.478; $Ca(IO_3)_2.6H_2O$, 0.295; and $CrCl_3.6H_2O$, 0.128.

[e]Sigma, St. Louis, MO, USA.

[f]Nitrogen-free extract (NFE) = 1000 – (protein + lipid + moisture + ash).

[g]Based on 23.4 kJ/g protein, 39.2 kJ/g lipid, and 17.2 kJ/g NFE.

[h]Protein/energy ratio (g/MJ).

Fish were hand-fed the experimental diets until apparent satiation 3 times daily (7 days a week) at 0700, 1200, and 1700 h, for 8 weeks. Feed consumption was recorded for each tank, based on the amount of feed that was taken by the fish. At the end of the experimental period, the fish were fasted for 24 h and the pooled weights of the replicates were recorded.

Sample collections and chemical analysis

At the start of the experiment, 30 fish from the original population were fasted for 24 h, euthanized, and stored at –20 °C for initial whole-body composition analysis. At the end of the experiment, 10 fish from each tank were randomly sampled and euthanized by overdose of ethylene glycol monophenyl ether, and kept frozen (–20 °C) for determination of their final whole-body composition. The analysis of diets and fish samples for crude protein, crude fat, moisture, and ash followed the standards outlined by the Association of Official Analytical Chemists (AOAC, 1995). Crude protein content (N × 6.25) was determined using the Kjeldahl method, after acid digestion, using an Auto Kjeldahl System (1030-Auto-analyzer, Tecator, Hoganos, Sweden). Crude lipid was determined by ether-extraction using a Soxtec extraction System HT (Soxtec System HT6, Tecator, Sweden). Ash was determined by muffle furnace at 550 °C for 24 h. Moisture content was measured by drying the tissues in an oven at 105 °C for 24 h and measuring the weight loss.

Performance parameters

The performance parameters including percentage weight gain (WG%), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), feed efficiency ratio (FER), feed intake (FI), protein intake (PI), and survival percentage were calculated as follows:

WG (%) = (final weight – initial weight)/initial weight × 100

SGR (% day^{-1}) = 100 × (ln final weight – ln initial weight)/time (days)

FCR = total feed intake (g)/weight gain (g)

PER = weight gain (g)/total protein intake (g)

FER = weight gain (g)/total feed intake (g)

FI = total feed intake/number of fish

Survival (%) = (final number of fish/initial number of fish) × 100

Statistical analysis

All statistical analyses were performed using the statistical package SPSS 14.0. In this experiment, each treatment (5 levels) was represented by 3 tanks (3 replicates). From each tank, 10 fish, representing 15% of the total number of fish per tank (30 fish per treatment), were randomly sampled. Descriptive statistics (means and standard deviations) of analysis results were calculated for each treatment. The homogeneity of variances of means was tested by Levene's test. When data were normally distributed, one-way analysis of variance (ANOVA) was calculated with percentage protein in the diet as a factor. When significant differences among treatments were found ($P < 0.05$), the means were compared using Duncan's multiple range test. Polynomial regression analysis was used to analyze the relationship between percentage weight gain and dietary protein level (Kim and Lee, 2005).

Results

Survival rate during the experimental period was 95% and above for all of the treatments. Analysis of fish growth performance (Table 2) indicated that fish fed a diet containing 42% protein had the highest weight gain and specific growth rate, and the lowest feed conversion ratio. According to the results, growth of kutum increased significantly ($P < 0.05$) with increasing dietary protein level from 32% to 42%, but there was a significant decrease ($P < 0.05$) in growth with increasing the dietary protein level from 42% to 52% (Table 2). The relationship between weight gain and dietary protein was best expressed using polynomial regression analysis ($y = 0.5467 x^2 + 45.533 x – 680.08$; $R^2 = 0.8088$); to ensure maximum weight gain of fingerlings, the optimal dietary protein level was determined to be 41.9% (Figure).

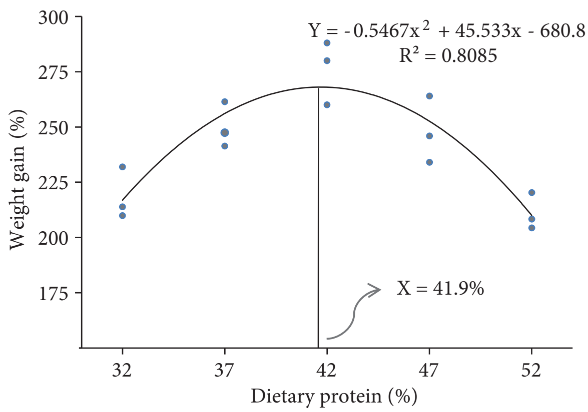

Figure. Relationship between percent weight gain and dietary protein levels for kutum fingerlings as described by second-polynomial regression. The vertical line in the middle of the graph represented the optimal protein level for kutum.

FCR was significantly affected ($P < 0.05$) by the dietary protein level. Increasing the dietary protein level from 32% to 42% decreased FCR, but, further, an

Table 2. Growth and feed utilization of kutum fingerlings fed diets containing different protein levels.

Dietary protein level (%)	Initial weight (g)	Final weight (g)	Weight gain (%)	FCR[2]	SGR[3]	FI[4]	PER[5]	FER[6]	Survival (%)
32	0.50 ± 0.11	1.59 ± 0.05^{c}	218.5 ± 11.7^{c}	2.34 ± 0.10^{a}	2.22 ± 0.07^{c}	2.56 ± 0.02^{a}	1.33 ± 0.06^{a}	0.42 ± 0.02^{c}	96.2 ± 1.25^{a}
37	0.50 ± 0.07	1.75 ± 0.05^{b}	250.0 ± 10.2^{b}	1.97 ± 0.02^{bc}	2.40 ± 0.05^{b}	2.45 ± 0.12^{a}	1.38 ± 0.01^{a}	0.51 ± 0.01^{ab}	97.08 ± 0.72^{a}
42	0.49 ± 0.09	1.88 ± 0.0.07^{a}	276.00 ± 14.4^{a}	1.85 ± 0.10^{c}	2.54 ± 0.07^{a}	2.55 ± 0.01^{a}	1.28 ± 0.07^{a}	0.54 ± 0.02^{a}	95.00 ± 1.2^{a}
47	0.50 ± 0.11	1.74 ± 0.07^{b}	247.9 ± 15.0^{b}	2.03 ± 0.11^{b}	2.39 ± 0.08^{b}	2.51 ± 0.02^{a}	1.04 ± 0.06^{b}	0.49 ± 0.02^{b}	95.8 ± 1.9^{a}
52	0.49 ± 0.07	1.55 ± 0.04^{c}	210.9 ± 8.3^{c}	2.34 ± 0.07^{a}	2.18 ± 0.05^{c}	2.47 ± 0.04^{a}	0.82 ± 0.02^{c}	0.42 ± 0.01^{c}	97.5 ± 1.2^{a}
P value		0.000	0.000	0.000	0.000	0.694	0.002	0.001	0.454
F value		24.17	26.12	15.05	28.14	0.565	8.11	12.33	0.362

[1]Values (expressed as mean ± SD, n = 3) with different letters in the same column are significantly different from each other ($P < 0.05$).
[2]Feed conversion ratio.
[3]Specific growth rate.
[4]Feed intake (g).
[5]Protein efficiency ratio.
[6]Feed efficiency ratio.

increase in dietary protein level to 52% significantly increased FCR.

FI ranged from 2.45 ± 0.02 to 2.56 ± 0.02 g/fish (Table 2) and different levels of dietary protein had no significant effect (P > 0.05) on feed intake. PER was significantly influenced (P < 0.05) by the dietary protein level. The lowest PER value was observed in diets containing 52% protein. PER did not show any significant difference (P < 0.05) among fish fed diets containing 32%, 37%, or 42% protein.

Whole-body composition of the fingerlings was determined at the start and end of the experiment (Table 3). The moisture content of *R. frisii kutum* was not significantly different among the experimental diets (P > 0.05). No significant differences were found in the whole-body lipid, protein, and ash contents of fish fed the different dietary protein levels.

Initial moisture content was significantly higher (P < 0.05) than the final moisture content in all of the experimental groups. Although overall variation in the whole-body protein content of the fish was low, fish fed the 47% protein diets had the highest body protein content (15.45 ± 0.20% live weight).

Discussion

According to Kim and Lee (2005), the broken line model and second-order polynomial analysis are the methods most often used to estimate dietary protein requirements of fish species. The second-order polynomial regression analysis appeared to be more appropriate in the present study because the weight gain (%) response was curvilinear. Furthermore, the R-squared value of the second-order polynomial regression analysis was higher than the R-squared value of the broken-line model. The optimal dietary protein level, estimated by polynomial regression analysis, for maximum growth of kutum fingerling was 41.9%, based on wet weight. Noverian et al. (2005) studied the effects of different dietary protein levels (25%, 30%, and 35%) on the growth performance in advanced kutum fry and found an optimal diet of 35% protein. This variation in reported protein requirements may have been caused by differences in fish size, water temperature, dietary energy level, and protein quality (Webster and Lim, 2002). The weight gain of kutum increased with increasing the dietary protein level from 32% to 42%, and decreased significantly (P < 0.05) thereafter, with further increases in dietary protein (Table 2). Similar results have been reported in other species (Santiago and Reyes, 1991; Yang et al., 2002; Mohanta et al., 2008). This is because in a high protein diet, part of the dietary protein is metabolized and converted to energy rather than used for growth (Mohanta et al., 2008). Dietary protein requirements of fish are closely related to dietary energy levels. If the energy level of a diet is low, dietary protein will be used to generate energy (Salhi et al., 2004). In general, an appropriate P/E ratio in the diet is important for optimum growth of fish (Tucker, 1992). The P/E ratios for optimum growth of several fish species ranged from 19 to 27 g/MJ (NRC, 1993). In the present study, maximum growth of kutum was obtained from the diet containing 42% protein and 19.1 MJ/kg energy, corresponding to a P/E ratio of approximately 21.7 g/MJ.

Table 3. Whole-body proximate compositions (% live weight basis) of kutum fingerlings before and after feeding trial.

Composition	Initial	Final						
		CP32	CP37	CP42	CP47	CP52	P value	F value
Moisture	76.2 ± 1.0	72.3 ± 1.3	72.2 ± 0.8	72.1 ± 0.7	72.0 ± 1.4	71.9 ± 0.7	0.991	0.066
Crude protein	12.3 ± 0.5	14.7 ± 0.6	15.2 ± 0.3	15.3 ± 0.5	15.4 ± 0.3	15.3 ± 0. 2	0.343	1.274
Crude lipid	8.4 ± 0.1	11.5 ± 0.4	11.7 ± 0.3	12.0 ± 0.4	11.7 ± 0.3	11.8 ± 0.8	0.860	0.317
Ash	2.8 ± 0.10	2.56 ± 0.06	2.45 ± 0.29	2.50 ± 0.16	2.40 ± 0.30	2.35 ± 0.21	0.804	0.401

Values are the mean ± SD of triplicate groups.
Values within the same row have no significant differences (P > 0.05).

In the present study, the FCR of the experimental diets varied from 1.80 to 2.33. This result is higher than that reported for kutum (2-10 g) by Noverian et al. (2005). The FCR means in their experiment varied between 1.45 to 1.65. This variation may be due to differences in initial weight, water temperature, lipid type and level, dietary protein, and energy content. The lowest dietary protein level (32%) produced the highest FCR, probably due to the intake of insufficient nutrient levels to promote growth. The best FCR was observed in fish fed the diet containing 42% protein. This result indicates that a diet containing 42% protein is optimal for kutum fingerlings.

All experimental diets were well accepted by kutum and the total feed intake was not affected by dietary protein level. Since the experimental diets used in the present study were isoenergetic, this suggests that kutum fingerlings controlled their feed intake in order to meet their energy requirements. It is generally known that fish eat to satisfy their energy needs (Webster and Lim, 2002).

In the present study, kutum fed the highest protein diets (47% and 52% protein) used dietary protein less efficiently, as reflected by the lower PER values recorded, than fish fed the low protein diets. In general, the protein efficiency ratio in carnivorous fishes decreases with high protein intake, as reported, for example, by Yang et al. (2003) and Salhi et al. (2004). This decrease is probably because dietary protein is used as an energy source when high protein diets are fed to fish (Jobling and Wandsvik, 1983; Yang et al., 2003). Mohanta et al. (2008) demonstrated that in silver barb fingerlings *Puntius gonionotus*, PER decreased significantly ($P < 0.05$) with an increased dietary protein level, which is what this study found in kutum as well. The inefficient conversion of protein to growth reflects the continued catabolism of protein for energy in the absence of any protein sparing effect at high protein levels. Protein sparing is the process by which the body derives energy from sources other than protein. In diets with high levels of protein, part of protein will be used to meet the energy requirement of the fish (Willson, 2002; Salhi et al., 2004).

Dietary protein levels did not significantly influence the final whole-body content of the *R. frisii kutum* fingerlings (Table 3). Whole body moisture and crude protein were not correlated to the dietary protein level. On the other hand, the lipid content of kutum increased with up to 42% protein and decreased with higher protein diets. However, carcass lipid levels for all of the treatments were not statistically different ($P > 0.05$). Our results on moisture and protein contents were consistent with studies of juvenile black sea bass, *Centropristis striata*, and tiger puffer, *Takifugu rubripes* (Alam et al., 2008; Kim and Lee, 2009). There was a tendency for whole-body moisture to decrease with increasing dietary protein fed to *R. frisii kutum*. Different results in other fish species like silver barb, big head carp, and Florida pompano (*Trachinotus carolinus*), were observed by Wee and Ngamsnae (1987), Santiago and Reyes (1991), and Lazo et al. (1998), respectively. The chemical composition of fish is different among various species. In addition, Shearer (1994) pointed out that the proximate composition of fish is influenced by both endogenous factors such as fish size and sex, as well as exogenous factors such as diet composition and the culture environment. For example, when the weight of a fish increases, the amounts of nutrients (protein, lipid, and carbohydrate) in the fish body will increase (Sutton et al., 2000).

Conclusion

Based on the results of this study, the maximum growth of Rutilus frisii kutum fingerlings was observed when fed the diet containing 42% protein (based on wet weight) with P/E ratio of approximately 21.7 g/MJ. From the polynomial regression analysis, the optimal dietary protein requirement of kutum was estimated to be 41.6%. We suggest that diets containing 41.6% crude protein and a P/E ratio 21.7 g/MJ can be more beneficial for kutum fingerling rearing. However, more detailed studies on the amino acid requirements of this species are required so as to formulate appropriate diets for this species.

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