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Research Article

Optimum dietary protein requirement for maximum growth performance of Caspian kutum (*Rutilus frisii*) juveniles

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Abstract

A 12-weeks feeding trial was conducted to estimate the optimum dietary protein level of Caspian kutum white fish as one of the highly important commercial species in the south of the Caspian Sea. The fish juveniles (0.59±0.05g body weight) were randomly distributed into 21 aquariums (30 fish per aquarium). The fish were fed with seven experimental diets containing 28, 32, 36, 40, 44, 48, and 52 % crude protein (CP).

Results showed that dietary protein levels significantly ($p<0.05$) affected growth performance and feed utilization of Caspian kutum juveniles. The fish fed a diet with 40% CP showed higher survival rate ($p<0.05$) than other groups. Feeding the fish with diets at 40-44% CP improved the weight gain (WG) and specific growth rate compared to 28%, 32%, and 52% CP diets. The feed conversion ratio and protein productive value were promoted by increasing the dietary CP level, while the protein efficiency ratio was decreased in diets containing high CP levels (44-52%). The fish receiving the protein supplemented diets showed higher protein and lipid content than the initial fish population. The results indicated that dietary CP level only affected the body protein content of the Caspian kutum juveniles ($p<0.05$). A broken-line regression calculated the protein requirement at 42.85-43.23 % based on the WG and PPV (protein productive value).

Keywords: Body composition, Caspian kutum, Dietary crude protein, Growth, Feed efficiency

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Introduciton

The aquaculture industry is now producing more than 114 million tons of various fisheries products (FAO, 2020) playing a considerable role in the supply of the required human food as well as contributing to food security in the world. Introducing new fish species to the fish farm can improve the supply, diversify aquaculture products and help promote the industry's efficacy in maintaining food security. The Caspian white fish or kutum (*Rutilus frisii Kutum* Kaminski, 1901) is a commercial fish of the Caspian Sea (Samavat *et al*., 2019) which is spread over a wide area ranging from the Kura river in Azerbaijan up to the Atrak river in Turkmenistan as well as in the Iranian side of the Caspian Sea (Mirzajani *et al*., 2016).

Like other fish under cultural conditions, the Caspian kutum need a balanced diet consisting of three basic nutritional elements such as proteins, lipids and carbohydrates (Craig and Helfrich, 2009; Hosseini Shekarabi *et al*., 2021a). Thus, the use of a diet comprising optimum protein level constitutes a major portion of this species' successful rearing and production plan. Protein is an important element that triggers the formation of different organs and tissues (Kaushik *et al*., 1995; NRC, 2011; Hosseini Shekarabi *et al*., 2021 a, b). Also it can help improve growth rate particularly among carnivorous fish (McGoogan and Gatlin, 1999). Therefore, it is imperative to secure adequate crude protein (CP) content within the fish diet (Siddiqui and Khan, 2009; Akpinar *et al*., 2012). Since the protein requirements of different fishes might vary according to fish age and species (Bowen, 1987; NRC, 2011), the optimum protein contents in the diet of various Cyprinid species have already been specified (Chang-an *et al*., 2014; Jin *et al*., 2015; Yun *et al*., 2015).

A limited number of studies on the effect of macronutrients, especially dietary CP levels, on the growth and body composition of the Caspian kutum fish juveniles, reported that the proper dietary protein is 35% for this species (Neverian *et al*., 2005; Mahmoodi *et al*., 2013). Indeed, an inadequate level of CP in fish practical diets can cause low fish farming performance, increased production cost and decline in water quality (Neverian *et al*., 2008a; Falahatkar *et al*., 2012). Therefore, the present study has been undertaken to assess the effects of dietary protein levels on the growth, feed utilization and body composition of kutum juveniles, together with an estimation of the required protein level in diets using the broken line regression method.

Material and methods

Diets preparation

Seven semi-purified diets containing graded levels of 28, 32, 36, 40, 44, 48, and 52% crude protein were formulated by Lindo Ver.6.1 (Neverian *et al*., 2005; Zahmatkesh and Karimzadeh, 2018) (Table 1). Gelatin, egg white and fish meal were used as protein sources in the diets. Upon weighing the ingredients, they were thoroughly mixed, added with distilled water to produce a paste form. The paste material was pelletized through a meat chopper machine with apertures 2mm in diameter. The dough obtained was dried in the oven at 60°C over-night. The dough was first formed into small-sized pellets and passed through sieves with mesh sizes ranging from 1 to 1.5 mm. The pellets were kept at -20°C until used.

Table 1: Formulation and proximate composition (% dry weight) (mean±SD) of experimental diets.

Ingredients	Dietary protein level (%)						
	28	**32**	**36**	**40**	**44**	**48**	**52**
Gelatin [a]	7	7	7	7	7	7	7
Egg albumin [b]	0.56	5.98	11.42	16.86	22.28	27.78	33.14
Herring fish meal [c]	20	20	20	20	20	20	20
Baker yeast [d]	10	10	10	10	10	10	10
Wheat flour [e]	36.42	30.96	25.48	20	14.54	9	3.58
Potato starch [f]	5	5	5	5	5	5	5
Dry milk [g]	4	4	4	4	4	4	4
Fish oil [h]	4	4	4	4	4	4	4
Sunflower oil [i]	7.42	7.46	7.5	7.54	7.58	7.62	7.68
Mineral premix [k]	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin premix [l]	3	3	3	3	3	3	3
Antioxidant [m]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Proximate composition							
Dry matter	92.3±1.0	92.4±1.1	92.2±0.9	92.6±0.8	92.4±1.1	92.5±0.8	92.4±1.1
Crude protein	28.4±0.7	31.8±0.9	35.7±0.2	40.5±0.9	44.6±0.6	48.0±1.1	51.8±0.4
Crude fat	16.8±0.9	16.9±0.5	16.8±0.4	16.9±0.2	17.0±0.3	16.9±0.4	16.9±0.2
ash	7.7±0.4	7.9±0.5	8.6±0.2	8.6±0.2	8.7±0.4	9.1±0.3	9.4±0.2
Crude fiber	5.1±0.2	4.2±0.2	2.3±0.2	2.3±0.1	1.8±0.3	3.9±0.4	1.3±0.2
NFE [n]	34.3±0.5	31.6±1.3	29.1±0.9	24.3±2.1	20.4±1.0	14.7±2.4	13.2±1.1
Energy (kcal/g)	3.37±.09	3.43±0.03	3.47±0.06	3.52±0.10	3.59±0.09	3.58±0.10	3.64±0.18

[a] Supplied by JAHANSHIMI Co., Ltd. (Tehran, Iran), 950 g/kg crude protein.
[b] Supplied by GOLPOODR Co., Ltd. (Golestan, Iran), 800 g/kg crude protein.
[c] Supplied by PARSKILKA Co., Ltd. (Mazandran, Iran), 600 g/kg crude protein, 110 g/kg crude lipid, 105 g/kg ash, 101 g/kg moisture.
[d] Supplied by PURETOP Co., Ltd. (Khozestan, Iran), 460 g/kg crude protein.
[e] Supplied by AZARGANDOM Co., Ltd. (Guilan, Iran).
[f] Supplied by TAKALVAND Co., Ltd. (Hamadan, Iran).
[g] Supplied by NUTRICIA Co., Ltd. (Netherlands),194 g/kg crude protein, 247 g/kg crude lipid.
[h] Supplied by PARSKILKA Co., Ltd. (Mazandran, Iran).
[i] Supplied by BAHAROIL Co., Ltd. (Tehran, Iran).
[k] Mineral premix (mg/kg of diet): $MgSO_4 \cdot 7H_2O$, 3600; KI, 20; $FeSO_4 \cdot H_2O$, 5200; $ZnSO_4 \cdot H_2O$, 3600; $CuSO_4 \cdot 5H_2O$, 500; $Na_2Se_2O_3$, 0.2; $MnSO_4 \cdot H_2O$, 3600; $CoCl_2 \cdot 6H_2O$, 15. Supplied by ISFAHAN MOKAMMEL Co., Ltd. (Isfahan, Iran).
[l] Vitamin premix (mg/kg of diet): retinyl acetate (2800 IU/mg), 40; cholecalciferol, 0.6; dl-_-tocopheryl acetate, 600; menadione, 60; thiamine hydrochloride,160; riboflavin, 220; pyridoxine hydrochloride, 160; vitamin B12, 0.4; ascorbic acid, 1000; folic acid, 20; biotin, 2; niacin, 600; calcium d-pantothenate, 640; inositol, 500. Supplied by ISFAHAN MOKAMMEL Co., Ltd. (Isfahan, Iran).
[m] BHT. Supplied by JAHANSHIMI Co., Ltd. (Tehran, Iran).
[n] NFE (nitrogen-free extract) = 100−(crude protein + crude lipid + ash + crude fiber + moisture).

Experimental animals and design

A total of 800 kutum fish juveniles were obtained from a state-owned fish hatchery center (Siahkal Fish Stock Enhancement and Propagation Center, Guilan Province, Iran). After being moved into 100L aerated fish tanks, were transferred to the Mirzakochak khan Centre for Fisheries sciences & technology. The fish were initially transferred in 2000 L tank and acclimated to rearing conditions for 10 days.

The juveniles were introduced into 21 glass tanks (aquaria, 50×35×40 cm) and reared for 12 weeks. Thirty fish (initial mean body weight 0.59±0.05 g) were randomly distributed into each tank (Ahmadian *et al*., 2015) with aeration supplied through an air pump (HAILEA, Aco-450, China).

Water quality, including temperature, dissolved oxygen, and pH was measured and recorded daily basis using digital multi meter (GENWAY 370, UK). During the rearing period, the average water temperature, dissolved oxygen, nitrite, nitrate and pH levels were estimated in different treatments at 20.57±1.90°C, 6.76±0.65 mg/L, 0.16±0.02mg/L, 3.80±0.35mg/L, and 8.18 ± 0.12, respectively. The daily photoperiod was set at 12-h light and 12-h dark. The daily water recirculation rate was 20% of the whole volume (12 L) in each tank. In addition, during the experimental period each tank was completely washed and filled with fresh water every 2 weeks.

The fish were fed three times a day (at 08:00, 13:00 and 20:00 h) by a feeding rate of 8% wet body weight. The weight and length of kutum juveniles were measured every two weeks for monitoring their growth and determining their daily feeding requirement per tank. After the feeding trial, fish were counted and weighed and the different indices such as weight gain rate (WGR), specific growth rate (SGR), condition factor (CF) and feed conversion ratio (FCR) were calculated.

At the end of the experiments, 5 juveniles were collected in each tank (from each treatment 15 juveniles) and kept at -20°C for carcass analysis. The proximate chemical analysis of fish carcasses were determined based on the standard international methods (AOAC, 1990). To determine the moisture, 5 g of the sample was placed in drier at 105˚C until constant weight (approximately for 24 h). Protein content was measured according to the Kjeldahl method after acid digestion by Kjeldahltherm (KBL20S, Gerhardt, Germany). Lipid measurement was carried out through the petroleum ether extraction and the use of Soxhlet-Extraktion system (B-810, Büchi, Netherlands) with fiber content specified using by glass crucibles and crude fiber extraction system (Foss Fibertec 1020 Hot and 1021 Cold extractor, Denmark).

Statistical analysis

Statistical analysis was done using a SPSS software package (18.0, SPSS Statistics, IBM, Chicago, IL, USA). After confirming the normality and homogeneity of variance, differences between means were tested for significance ($p<0.05$) by the one way ANOVA analyses. Tukey HSD's multiple-range test was used to determined significant differences among means. Optimal protein requirement in the diet was calculated using broken-line regression model (Robbins *et al*., 2006) and SAS version 9 software package.

Results

The survival rate of kutum fish juveniles fed experimental diets at different protein levels is presented in Figure 1. After 12 weeks of the feeding trial, the survival rate among kutum juveniles fed the 40% diet showed to be higher (97.78%) than that of in other treatments, specifically those fed the 28% diet (88.33%) ($p<0.05$). The growth performance of juvenile kutum fish is indicated in Table 2. Final weight and fish condition factor in all treatments were not significantly affected by the dietary protein level ($p>0.05$). Weight gain rate and special growth rate of kutum juveniles fed the 28, 32 and 52% crude protein were significantly lower than those of the fish fed the 36, 40 and 44% CP diets ($p<0.05$).

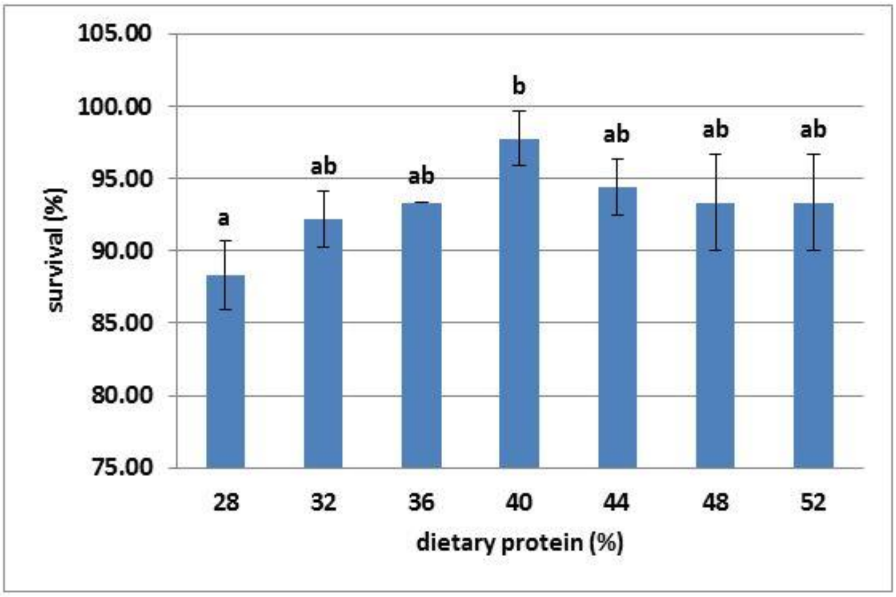

Figure 1: Survival of *Rutilus frisii kutum* juveniles fed diets containing various levels of protein after 12 weeks rearing period.

Parameters of nutrient utilization (FCR, PER and PPV) were significantly affected by the levels of dietary protein (Table 2, $p<0.05$). FCR among juveniles tended to improve in line with an increase in protein content of diets up to 40% protein level ($p<0.05$). PER significantly decreased with incremental levels of dietary protein ($p<0.05$). PER of fish fed the 32% protein diet was significantly higher than those of fish fed the other experimental diets. Protein productive value (PPV) was progressed with increasing dietary protein level and was found to be the highest for fish fed 40% CP ($p<0.05$). No significant difference were observed in feed intake among fish fed the experimental diets.

The production rate in experimental treatments was affected by protein content of diets to the extent that a rise in protein level was associated with significant production increase (p<0.05). The lowest production (16.42±1.30g in tank) and the highest production level (23.86±1.05g in tank) were estimated in 28% and 40% diets respectively (Table 2).

Table 2: Growth performance and feed utilization (mean±SD) of *Rutilus frisii kutum* juveniles fed diets with different levels of crude protein for 12 weeks.

Parameters	Dietary protein level (%, dry matter)						
	28	32	36	40	44	48	52
Initial weight (g)	0.61±0.00	0.62±0.01	0.57±0.03	0.60±0.02	0.57±0.03	0.58±0.03	0.61±0.03
Final weight (g)	1.31±0.01	1.39±0.03	1.34±0.07	1.42±0.03	1.35±0.07	1.35±0.08	1.37±0.07
Weight gain (g)[1]	0.70±0.01[a]	0.78±0.02[ab]	0.77±0.03[ab]	0.83±0.02[b]	0.78±0.04[ab]	0.77±0.05[ab]	0.76±0.04[ab]
Weight gain rate[2]	114.75±2.32[a]	125.94±1.22[b]	134.53±1.19[c]	138.57±2.18[c]	138.29±2.34[c]	133.28±2.56[c]	125.85±1.85[b]
Specific growth rate (%/day)[3]	0.91±0.02[a]	0.97±0.01[b]	1.01±0.01[c]	1.03±0.02[c]	1.03±0.01[c]	1.01±0.02[c]	0.97±0.01[b]
Feed intake (g/fish)[4]	2.30±0.03	2.01±0.02	2.07±0.08	2.04±0.13	2.00±0.18	2.11±0.21	2.27±0.15
Feed conversion ratio[5]	3.28±0.03[b]	2.58±0.06[a]	2.70±0.10[ab]	2.47±0.11[a]	2.56±0.35[a]	2.73±0.24[ab]	2.98±0.24[ab]
Protein efficiency Ratio[6]	1.09±0.01[de]	1.21±0.03[e]	1.03±0.04[cd]	1.01±0.04[cd]	0.90±0.12[bc]	0.77±0.08[ab]	0.65±0.05[a]
Protein productive value (%)[7]	204.72±90.50[ab]	267.86±96.75[ab]	294.11±133.98[ab]	409.86±82.58[b]	343.64±114.22[ab]	215.58±69.41[ab]	122.88±2.55[a]
Condition factor (g/m³)[8]	0.82±0.02	0.88±0.05	0.88±0.05	0.81±0.06	0.89±0.11	0.82±0.10	0.79±0.04
Production (g/tank)[9]	16.42±1.30[a]	20.04±0.32[b]	20.33±0.80[b]	23.86±1.05[c]	21.26±1.49[bc]	20.47±1.63[b]	20.12±0.58[b]

Values within the same row with different letters are significantly (p<0.05) different.
[1] Weight gain: final mean weight − initial mean weight.
[2] Weight gain rate: 100×(final mean weight − initial mean weight) / initial mean weight.
[3] Specific growth rate: 100×[Ln (final mean weight)–Ln (initial mean weight)] / days of the experiment.
[4]Feed intake (dry matter, g/fish) = total feed fed (g)/fish.
[5] Feed conversion ratio: g dry feed / g weight gain.
[6] Protein efficiency ratio: g weight gain / g protein fed.
[7] Protein productive value: 100×g protein gain / g protein fed.
[8] Condition factor: 100×fish weight (g) / fish length (cm) ^{3}.
[9] Production: final mean biomass (g) – initial mean biomass (g).

According to the broken-line regression analysis of weight gain (%) and protein production value (%) against dietary protein content, the optimum protein requirement of the kutum diet was estimated at 43.23% and 42.85% respectively (Figs. 2 and 3).

The result of carcass composition analysis of experimental fish feeding on diets with different protein levels is indicated in Table 3. Initial carcass composition of fish contained more moisture than final carcass composition of fish but relatively lower protein and lipid contents, regardless of experimental diets.

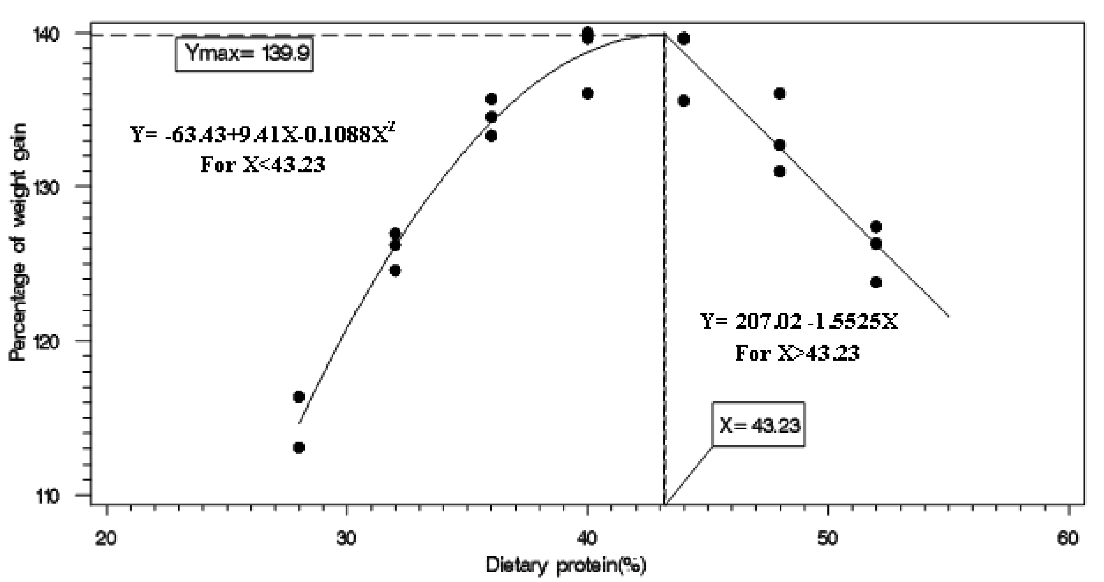

Figure 2: The quadratic broken-line model fitting weight gain (%) to dietary protein levels in *Rutilus frisii kutum* juveniles fed the experimental diets (R^2 = 0.9621). Ymax, maximum growth rate; X, a protein level required for Ymax. Each graphic point represents the average value of each tank.

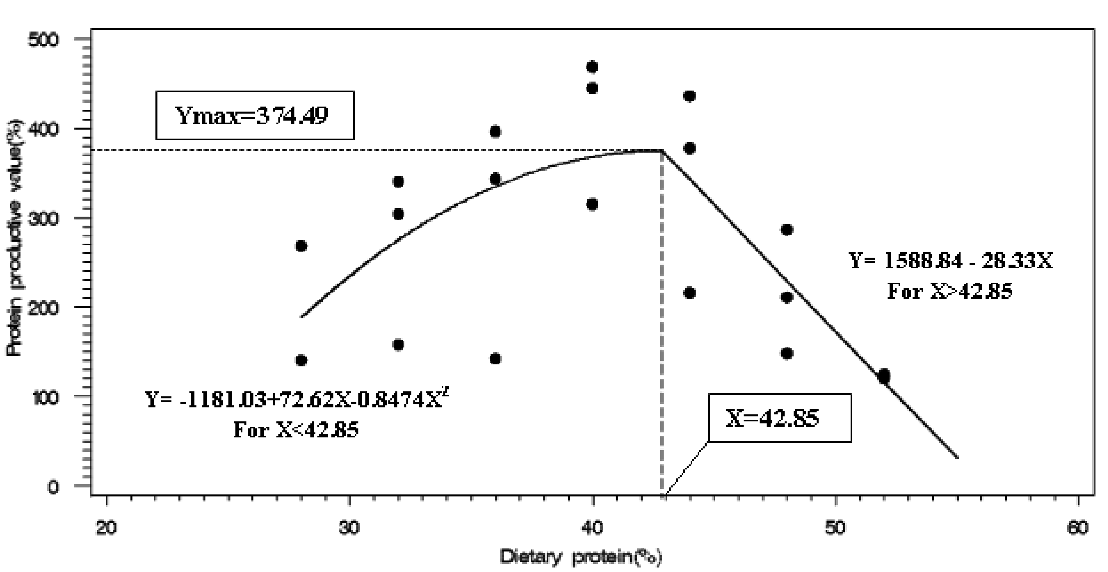

Figure 3: The quadratic broken-line model fitting protein productive value (%) to dietary protein levels in *Rutilus frisii kutum* juveniles fed the experimental diets (R^2 = 0.8163). Ymax, maximum growth rate; X, a protein level required for Ymax. Each graphic point represents the average value of each tank.

Moisture level in the carcass of kutum juveniles declined with increasing of diet protein but no significant difference was observed in the dietary treatments (p>0.05). Crude protein content in the carcass of kutum juveniles was affected by dietary protein level (p<0.05). The protein content in the carcass of fish (17.28±0.52) fed the 40% protein showed to be significantly higher than those in the fish receiving 28%, 32% and 52% dietary proteins (p<0.05). Although lipid variation in the carcass of kutum juveniles was similar to that of protein, there were no significant differences in the amount of such index across treatments involving the use of diets of varying protein levels ($p>0.05$). The highest lipid content (12.6±1.20) was detected in the carcasses of kutum juveniles fed the 44% diet. The result of the present study showed that the protein level of diet does not have any impact on the ash content of kutum fish juvenile's carcass ($p>0.05$).

Table 3: Whole-body compositions (fresh-wt. basis) (mean±SD) of *Rutilus frisii kutum* juveniles fed different dietary protein levels for 12 weeks.

Dietary protein level (%)	Body composition			
	Moisture %	**Protein %**	**Lipid %**	**Ash %**
Initial[1]	69.48±1.72[b]	13.99±0.42[a]	8.71±0.99 [a]	4.05±0.51
28	67.61±0.58[ab]	15.27±0.60[ab]	10.39±0.21[ab]	3.91±0.45
32	67.27±0.42[ab]	15.67±0.63[ab]	10.49±0.89[ab]	3.85±0.65
36	67.21±0.29[ab]	16.33±1.11[bc]	10.52±1.46[ab]	3.85±0.38
40	65.76±0.30[a]	17.28±0.52[c]	11.42±0.66[ab]	3.84±0.44
44	65.55±1.15[a]	16.94±0.94[bc]	12.06±1.20[b]	4.12±0.36
48	66.51±0.54[a]	16.10±0.53[bc]	11.51±1.25[ab]	3.93±0.39
52	67.10±1.04[ab]	15.40±0.13[ab]	10.06±1.09[ab]	3.95±0.36

Values within the same column with different letters are significantly different ($p<0.05$).
[1]The initial fish stock before starting the experiment.

Discussion

After 12 weeks of the feeding trial, the survival of experimental fish averaged 93.25%. The survival was significantly different between the diets containing 28 and 40% CP. Survival rate of fish under trial condition depends on many factors including water quality, fish density, sanitation of the rearing environment and more specifically the quality and quantity of fish feed. In the present study, increasing dietary protein level up to 40% resulted in relative improvement of survival rate in juvenile kutum fish whereas the lowest survival rate was noticed in fish fed the 28% diet. This might account that diets of lower than 30% protein level are not suitable for kutum juveniles. However, many researchers reported that the survival of other fish such as catfish (Khan, 1993; Deng, 2011) and cyprinids (Jin *et al*., 2015; Suharmili *et al*., 2015) have shown to be less affected by protein level.

In this study, growth parameters (*i.e.* WG, WGR, and SGR) tended to improve significantly in line with an increased diet protein and the highest growth performance was obtained at 40% protein level. A number of researches had already suggested the positive effects of protein levels on fish growth parameters (Steffens, 1981; Li *et al*., 2010; Jin *et al*., 2015), while other studies reported a reverse relationship between protein levels of diet and weight gain in common carp, *Cyprinus carpio* (Cho *et al*., 2001) and Indian carp, *Cyprinus catla* (Parveen and Sheri, 1994; Satpathy *et al*., 2003). Such a different result might have been due to optimizing dietary protein level to growth and supplementation of energy requirements through carbohydrates and lipids (Caballero *et al*., 2002). The optimum protein content in a diet relies upon sufficient provision of energy since part of the protein is used as energy reserve when fish feed energy is insufficient (Lovell, 1991; Salhi *et al*., 2004). Therefore, there should be a suitable protein / energy ratio in the diet to effectively utilize the protein. Jin *et al*. (2015) reported that grass carp (*Ctenopharyngodon idella*) juveniles fed the 40% protein diet enjoyed the best growth performance which is similar to the result of the present study.

In this study, the highest FI and FCR were observed among kutum juveniles fed 28% protein diet. The greater feed intake in dietary treatment containing lower protein and their higher FCR level might have been due to the fact that the nutrients in diets should be commensurate with tissue formation and repair requirements and metabolic functions of the fish body (Winfree and Stickney, 1981). When fish fed diets with sub-optimal protein contents, they tend to consume higher amounts of feed so as to meet their protein and energy requirements for growth and development (Gaylord and Gatlin, 2001; Wang et al., 2016). Similar to the results reported from other studies (Steffens, 1981; Tuan and Williams, 2007), the findings of the present study indicated FCR improvement upon increasing diet protein up to 40%. This might be attributed to sufficient provision of energy and its effective use for inducing maximum growth of juveniles in these treatments.

In this study, an increase in dietary protein level decreased PER and raised PPV. Such a phenomenon has also been reported in other fish species (Dabrowski, 1977; Lee *et al*., 2002; Suharmili *et al*., 2015; Jiang *et al*., 2016). Generally, in carnivorous fish, PER is inversely correlated linearly with protein (Yang *et al*., 2003; Salhi *et al*., 2004). A dietary protein content above of required optimum level is used as an energy source (Shyong *et al*., 1998; Yang *et al*., 2003) which might induce increased enzymatic activity that breaks down amino acids in hepatopancreas, triggering greater nitrogen diffusion and thus reducing PER/PPV (Shimeno *et al*., 1981). The increased PPV associated with a rise in protein level (up to 40%) and P/E (up to 27.4 g/Mj) might have been caused by the formation of a proper P/E ratio within the diet and subsequently suitable protein retention in the body of experimental fish. As indicated in earlier studies, fish need a specific P/E ratio in their feed to gain an optimum growth (Tucker, 1992; Neverian *et al*., 2008b) and P/E ratio for optimum growth defined for fish species ranges in 19-27 g/Mj (NRC, 2011).

In the present study, kutum fish juveniles with better growth rates enjoyed also the highest CF as was reported in grass carps by Jin *et al*. (2015). Higher CF values represented the appropriate nutritional and health status of fish and their suitable growth rate which is an ideal in aquaculture (Ayode, 2011; Castro *et al*., 2016).

Estimating of yield amount is considered as a foundation of economic production estimation in aquaculture operations (El-Saidy and Gaber, 2005). In this study, fish production, estimated as per biomass and survival rate, began to rise when protein level in the diet was increased. Since kutum fish juveniles survival and growth rate were influenced by protein diet, it was evident that production increase could, likewise be expected.

Protein and lipid contents in the body of kutum fish fed the diet with high protein levels were significantly higher than the initial fish and those fed by a diet containing lower protein, whereas moisture level declined with an increase in protein content. In addition, protein in the body of fish feeding on 40% dietary protein turned to be significantly greater than those in treatments using feeds of 28, 32 and 52% protein levels. In agreement with this study, the whole body protein in cyprinids such as lemon fin barb hybrid, *Hypsibarbus wetmorei* ♂×*Barbodes gonionotus*♀ (Suharmili *et al*., 2015) and Mahseer, *Tor putitora* (Hossain *et al*., 2002) began to rise with an increase in dietary protein level. The highest whole body protein was specified in the body of grass carp juveniles fed 40% diet (Jin *et al*., 2015) which is in line with the present research.

In the present study, the highest protein and lipid contents were observed among juveniles fed diets containing 40 and 44% protein, respectively. This might reflect adequate protein level and ultimately, suitable P/E ratio that induces maximum protein retention in body. It seems that a high protein level in diet (more than 44% in diet) results in extra energy generation. It has been indicated in previous research that excess energy in diet might entail the production of fat fish, decreased feed intake and could deter other feed ingredients from being utilized (Shiau and Lan, 1996).

The whole-body moisture of the experimental fish was negatively affected by increased protein levels in the diet, giving results similar to other research (Kim and Lee, 2005; Alam *et al*., 2008). The moisture decline could have been due to increased lipid in juveniles' bodies. The overall body moisture and lipids of juveniles remained nearly constant (Deng, 2011), parallel with reports often suggesting a reverse relationship between these two items in fish (Marais and Kissil, 1979; Afzal Khan *et al*., 2003).

The Increased protein level is associated with elevated fish feed cost, so that diets containing high protein level is known as an important restraint by many fish farmers, diminishing fish farming productivity. On the other hand, insufficient provision of protein and energy supply in feed might, apart from increasing fish feed production costs, diminish water quality due to feeding waste (Lee *et al*., 2002). Therefore, determining the protein requirement in the fish diet is necessary for promoting of feed efficiency and securing profitability in fish rearing operations. According to broken line analyzing, the optimum protein levels for maximum

PWG and PPV obtained 43.23% and 42.85% respectively. These values stand higher than the optimum protein levels for the other cyprinids species such as *Hypophthalmichthys nobilis* (30%) (Santiago and Reyes, 1991), lemon fin barb hybrid (34.6%) (Suharmili *et al*., 2015), *Catla catla* (Dars *et al*., 2010) and *Leptobarbus hoevenii* (Farahiyah *et al*., 2017), but that is almost equal with protein requirement of grass carp (Dabrowski, 1977; Jin *et al*., 2015). However, the required protein level in diet might vary according to size and species of fish, the feed formulation, rearing condition, water temperature, and more specifically, the energy level of diet as well as protein quality (Elangovan and Shim, 1997).

Based on the results obtained here, the optimum protein requirement in the diet of the Caspian kutum fish juveniles stands higher than that of other cyprinid species, suggesting that diets with high protein contents need to be used for maximum growth of this species. Although the estimated optimum protein level of the diet varied from 42.85 to 43.23%, it would be safe to suggest a 40% protein in any practical diet for rearing the kutum juveniles since it has caused a similar growth performance and the best protein retention rate. However, more studies need to be done on quality and sources of diet protein, energy requirement and P/E ratio aimed to devise a practical cost-effective diet for such species.

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