Optimum dietary protein requirement of Paradise fish, *Macropodus opercularis* based on growth and reproduction performances

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Received: February 2020 Accepted: June 2020

Abstract

The aim of this study was to assess the effects of dietary protein levels on growth and reproduction performance of paradise fish, *Macropodus opercularis.* A total of 540 fish (0.5±0.01 g) was offered one of six experimental diet comprising different protein levels (i.e., 25, 30, 35, 40, 45 and 50 %). Diet of 40 % crude protein produced significantly higher mean weight gain, daily growth rate, and specific growth rate and feed conversion ratio ($p \leq 0.01$). The analysis made based on the second order polynomial regression curve *($R^2=0.79$)*, revealed that optimum protein levels based on fish SGR and FCR were 40 and 45 %, respectively. The average total egg production increased with increasing dietary protein levels up to 45 %. There was no significant difference in the egg diameter among groups, but egg hatchability was significantly higher (96%±2) in the fish fed diet containing 45% protein. The highest and lowest GSI was obtained in the 45 and 25% dietary protein, respectively. Fecundity was highest in the fish fed diet containing 45% (107.4±12.1) and followed by 50 (91.4±8.5) and 40 (68.5±2.6) % dietary protein. There were no significant effects of dietary protein level on egg biochemical composition and amino acid profile. The results suggest optimum dietary protein levels of 40 and 45 % for maximum growth and reproduction performance in paradise fish, respectively.

Keywords: Paradise fish, Reproduction, Growth, Amino acids, Protein levels, Egg diameter

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Introduction

Ornamental fish trade has an important role in global economic, as several hobby fish species are annually traded in world (Hoseini et al. 2015; Hoseini and Tarkhani 2013). The knowledge of nutritional requirement in ornamental fish species is essential for sustainability of this industry (Masrizal et al. 2015). However, the nutritional information of these species is based on the results gained from non-ornamental species (Velasco-Santamaría and Corredor-Santamaría 2011). Compared with edible fish industry, the ornamental fish feed production has lagged. In addition, despite the importance of this industry, few data are available on the nutritional requirement of many ornamentals and accordingly, no specifically feed designed for ornamental fish species (Tamaru et al. 2001) . Recently, the food-fish diets are broadly being used for ornamentals from grow-out to broodstock stages (Garcia-Ulloa and Gomez-Romero 2005). However, the nutritional requirement of fish is varying at different life stages. Thus, there is a need to develop diets for ornamental fish to the requirement of ornamental fish at various life stages (James and Sampath 2004).

A balanced diet provides are macronutrients and micronutrients and secures fish efficient growth and well-being (Hoseini et al. 2017). There is a growing interest in applying specialized diet in ornamental fish industry. In this regard, studies directed toward protein nutrition in fresh water ornamental fish have shown protein requirements for growth range from 30% up to 50% in omnivores and carnivores, respectively (Hardy 2003). Protein is an important part of diets in fish, including ornamental one, which affect fish growth and production cycle. Optimum dietary protein provides required levels of amino acids for maximum growth rate and feed efficiency, thus brings maximum profit to fish farmers (Hoseini et al. 2018). On the other hand, reproductive performance and seed production of fish are directly related to broodstock nutrition and exogenous nutrient provides the crucial constituents mandatory for gonad development and quality seed production (Izquierdo et al. 2001). Although several species have been subjected to test their protein requirement during broodstock stages including Angelfish (Shelar et al. 2014), guppy, (Kithsiri et al. 2010), fighting fish, (James and Sampath 2003), and dwarf gourami (Shim et al. 1989), dietary protein requirement have remained unidentified in most ornamental species, yet (Afzal Khan et al. 2005). Moreover, it has been demonstrated that optimum dietary protein levels differ based on fish optimum growth rate, maximum growth rate or reproduction performance (Chong et al. 2004; Mithun et al. 2019).

Gouramies are among the freshwater ornamental species that include very popular paradise fish, *Macropodus opercularis.* This species is a labyrinth fish that is able to breathe atmospheric oxygen using its modified gills. The fish spawns in bubble nests, constructed and maintained by the male, who is

responsible for rearing the eggs and larvae. Although Villars and Davis (1977) described the reproductive behavior in Paradise fish, but there is a gap in the nutrient requirement of this species, especially in broodstock phase. The present study was conducted to investigate the effect of different protein levels on growth and reproductive performance of paradise fish.

Material and methods

Experimental diets

Ingredients were analyzed for proximate composition and the experimental diets were formulated using Animal Feed Optimization Software (AFOS). Experimental diets were produced at FEDAR Ornamental Fish Feed Company under fully automatic extrusion line with the capacity of 100 kg/hr. Six is oenergetic diets were formulated to contain 25, 30, 35, 40, 45, and 50 % crude protein (Table 1).

Table 1: Ingredients (g kg^{-1}) and proximate analysis results for all experimental diets.

Ingredients	Dietary protein (%)					
	25.0	**30.0**	**35.0**	**40.0**	**45.0**	**50.0**
Fish meal	5.5	75.5	82.5	11.6	15.8	18.7
Blood meal	4.8	7.0	12.0	14.0	15.0	18.0
Poultry by-product	5.0	8.0	8.0	10.0	13.0	16.0
Soybean meal	20.0	20.0	20.0	20.0	20.0	20.0
Wheat flour	20.0	20.0	20.0	20.0	18.6	10.5
Soybean oil	8.0	6.96	6.87	6.13	5.2	4.4
Corn gluten	1.0	1.0	1.0	1.0	1.0	1.0
Met	2.6	2.6	2.6	2.6	2.6	2.6
Lys	1.3	1.3	1.3	1.3	1.3	1.3
Na-Cl	2.0	2.0	2.0	2.0	2.0	2.0
Premix[1](vit+min)	5.5	5.5	5.5	55	5.5	5.5
Cellulose	24.3	180.9	124.8	58.7	0.0	0.0
	Chemical composition (%)					
Crude protein	25.4	30.1	35.2	40.3	45.1	50.1
Crude fat	10.3	10.4	10.4	10.4	10.1	10.2
Crude fiber	24.2	21.2	21.2	21.7	22.0	20.6
Crude ash	21.8	32.8	35.9	41.5	47.8	52.5
NFE	59.0	53.3	48.0	42.3	37.0	31.7
GE[3](Mj/kg)	20.2	20.4	20.6	20.8	21.0	21.3

[1]Vit premix (per kg diet): vit c, 40 mg; vit A, 2000 IU; vit D_3 150 IU; vit E, 150 mg; vit B_1, 75 mg; riboflavin, 20 mg; vit B_6, 12 mg; D-calcium pantothenate 20 mg; nicotinic acid, 30 mg; biotin 1.0 mg; inositol 15.3 g; choline 20 g. Mineral (per kg diet): Fe, 20 mg; Zn, 40 mg; Se, 0.2 mg; Cu, 5 mg; I, 30 µg; Co, 50 µg; Mg, 30 mg; P, 600 mg; K, 800 mg.

[3]Moisture of diets (6.9-7.2); mean values from three replicates; gross energy calculated based on 23.6, 39.5 and 17.2 kj/g for protein, lipid and carbohydrate, respectively.

Fish and maintenance

A total of 540 paradise fish (0.5±0.01 g) were collected from hatchery-bred brooders and divided into 18 aquaria (35 L) as six triplicate groups (30 fish per aquaria). Each group was offered one of the aforementioned diets based on 3% of biomass per day (divided into three meals). Waste materials were daily removed from the aquaria and 25% water renewal was daily performed. Continuous aeration was applied using two air blowers to provide the dissolved oxygen in the aquaria water. Water temperature 23 ºC, pH: 7.3, DO: 7.8 ppm and total hardness: 18dH, were measured as described by APHA (2005). After 16 weeks feeding, the fish growth parameters were calculated as follow (Mirghaed et al. 2019):

Feed conversion ratio (FCR): feed intake / weight gain

Weight gain (WG): 100 × [(final weight – initial weight)/ initial weight]

Specific growth rate (SGR): 100 × [(ln final weight – ln initial weight)/ days of experiment]

Spawning procedure

The morphological differentiation of brooders was determined based on the basis of the belly diameter and the shape of the dorsal fin. Females have a round dorsal fin and higher belly diameter while males have a picked dorsal fin. As soon as fish showed sign of morphological differentiation, 10 male and 10 females were separately kept in two holding aquaria (80-L) and fed with experimental diets three times per day. One male was randomly selected from this holding aquarium and introduced to each experimental aquarium (one male per aquarium) in each treatment. After preparation of the foam nest building by males (using plastic sheets 30×30 cm^2 sticking to each other at the water surface), females were introduced to each aquarium. Therefore, one pair of fish individually was placed in an aquarium (80- L) with an allocation of 10 replicates aquarium per experimental diet). After spawning, the buoyant eggs, which rose to the water surface, were collected by filtered mesh (200 µm). Oocyte, yolk sac, and egg, diameter was measured using micrometer of a light microscope. The eggs collected from every individual spawning gourami, were used to assess the fertilization and hatching rate. The fertilization and hatching rate testing were carried out using a 15-L aquarium containing 1000 eggs 12 hours after incubation (three times per replicate). The individual weight of females was measured and individual brooders from every diets treatment were sacrificed to assess the gonad osomatic index (GSI). The method for breeding procedure is described in Reich (2018). In order to investigate the effect of dietary protein level on fry quality, freshly sampled fry was immediately frozen at -20ºC for

measurement of total length, dry weight and proximate analysis. Reproduction performance was calculated as follow:

GSI: [ovary weight / body weight] × 100
Absolute Fecundity: total egg produced per female
Relative fecundity: total egg produced / female body weight
Larval length: was measured using micrometer of a light microscope
Egg diameter: was measured using micrometer of a light microscope
Hatching rate: [total number of larvae / total number of fertilized eggs] × 100
Fertilization rate: [total number of hatched eggs / total number of eggs produced] × 100

Diets and tissue proximate and amino acid analysis

At the end of the experiment, nine fish were caught from each treatment for analysis. Crude protein, lipid, moisture, and ash of whole-body, diets and ovaries were determined using (AOAC 2005). In brief, samples of diets and fish were dried to a constant weight at 135 ºC for 2 h to determine the moisture content. Ash was generated by incineration using muffle furnace at 550ºC for 3 h. Crude lipid was determined by Soxhlet extraction unit using a soxtec system and crude protein content was analyzed by the Kjeldahl method (N×6.25) after acid digestion. All samples were analyzed for amino acid composition using method described by (Rutherfurd and Gilani 2009) High-Performance Liquid Chromatography (HPLC) system (Breeze, Water Corporation, Milford, MA, USA) according to the manufacturer's instruction.

Statistical analysis

The growth and reproduction data as well as biochemical results samples were analyzed statistically using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test via SPSS software. Quadratic regression model was used to determine optimum dietary requirement. Significant differences were based on the $p \leq 0.05$ level. The data are presented as means ±SD.

Results

Growth parameters of paradise fish in response to different dietary protein levels are given in Table 2. Fish fed diet containing 40% crude protein produced significantly ($p \leq 0.01$), higher mean weight gain. The lowest mean weight gain was recorded in 25 and 30% CP-fed fish. Moreover, the highest DGR, SGR and FCR ($p \leq 0.01$) were recorded at the fish fed diet containing 40%, while the least productive diets were 25 and 30% crude protein. The growth parameter of 50% CP-fed fish was higher than the diet containing 25 - 35%, however it was less efficient than the diet containing 40 and 45% crude protein. Overall, data showed the crude protein for optimum growth rate in paradise fish is ranged from 40 to 45%.

Table 2: Growth performance of paradise gourami after 16 weeks of feeding with diets containing different protein (%) levels. Values with the same superscript within the same column are not significantly different (Duncan test, $p \geq 0.01$; n = 3).

Dietary protein (%)	Initial weight (g)	Final weight (g)	WG (%)	SGR (%/d)	FCR
25.0	0.555±0.001^{a}	4.77±0.16^{d}	88.3±0.44^{c}	1.88±0.03^{d}	1.20±0.01^{c}
30.0	0.559±0.040^{a}	4.84±0.05^{d}	88.4±0.20^{c}	1.90±0.01^{d}	1.17±0.10^{c}
35.0	0.563±0.006^{a}	5.30±0.02^{c}	89.3±0.13^{b}	1.98±0.00^{b}	1.04±0.20^{b}
40.0	0.596±0.010^{a}	5.96±0.01^{a}	90.4±0.30^{a}	2.09±0.01^{a}	0.96±0.30^{a}
45.0	0.561±0.001^{a}	5.63±0.01^{b}	90.0±0.02^{a}	2.05±0.00^{a}	0.96±0.01^{a}
50.0	0.572±0.010^{a}	5.34±0.01^{c}	89.2±0.33^{b}	1.98±0.01^{b}	0.99±0.05^{b}

Quadratic regression models showed that optimum dietary protein levels were 40 and 45% based on SGR (R^2=0.79) and FCR (R^2=0.92), respectively (Fig. 1).

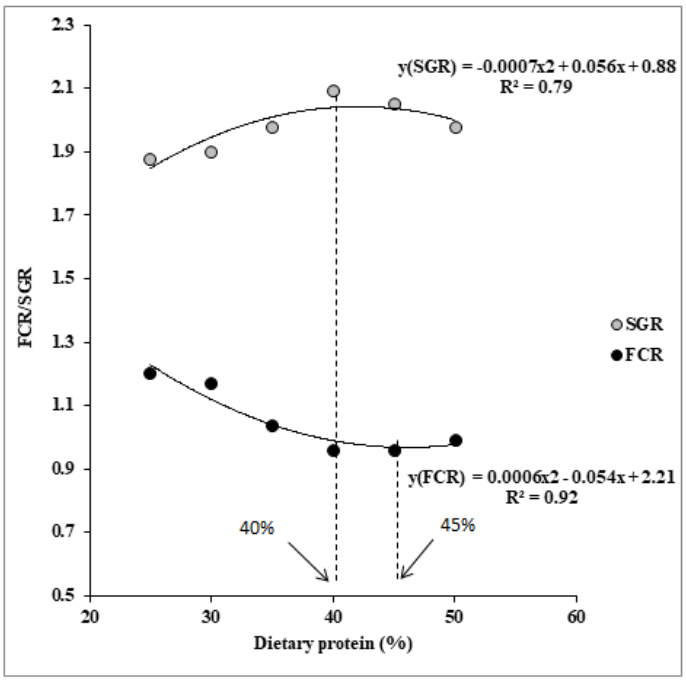

Figure 1: Quadratic regression model for optimum dietary protein calculation based on SGR and FCR in paradise fish.

Results of the fish reproductive performance are given in Table 3. Total egg production increased with increasing dietary protein levels up to 45%. The highest egg production ($p \leq 0.01$) was obtained with a dietary of 45 % (605±15), followed by 50% (488±10.2) and 40% (409.3±16.7). The highest and lowest GSI's were obtained in the fish fed with diets containing 45 and 25% protein, respectively. The highest fecundity was observed in the fish fed with the diet containing 45% crude protein (107±12.1), followed by fish fed diets containing 50 (91.4±8.50) and 40 (68.5±2.60) % dietary protein.

Table 3: Reproductive performance of paradise gourami after 16 weeks of feeding with diets containing different protein (%) levels. Values with the same superscript within the same column are not significantly different (Duncan test, $p \geq 0.01$; n = 3).

Dietary protein (%)	Absolut fecundity	Egg diameter	Hatching rate	Larval length	Fertilization rate	GSI	Relative Fecundity
25.0	276±25.1^{d}	1.10±0.05^{a}	89.3±1.50^{b}	3.8±0.05^{b}	89.6±0.50^{a}	0.78±0.10^{c}	58.1±7.10^{c}
30.0	293±75.0^{c,d}	1.20±0.10^{a}	89.6±4.10^{b}	3.9±0.20^{b}	90.0±0.50^{a}	0.94±0.03^{c}	60.4±6.70^{c}
35.0	343±35.1^{c,d}	1.40±0.30^{a}	90.0±1.00^{b}	4.0±0.20^{a,b}	91.0±1.00^{a}	1.10±0.10^{c}	64.7±6.40^{c}
40.0	409±16.7^{b,c}	1.30±0.05^{a}	93.3±1.50^{a}	4.1±0.10$^{a, b}$	92.0±1.00^{a}	1.60±0.10^{b}	68.5±2.60^{b,c}
45.0	606±15.0^{a}	1.45±0.05^{a}	96.0±2.00^{a}	4.5±0.05^{a}	92.0±1.00^{a}	2.10±0.20^{a}	107±12.1^{a}
50.0	489±10.2$^{a, b}$	1.30±0.10^{a}	93.0±2.00^{a}	4.0±0.10$^{a, b}$	91.0±0.50^{a}	1.80±0.10^{a,b}	91.4±8.50^{a,b}

There was no significant difference in the egg diameter among different dietary treatments. Hatching rates of the fish fed diets containing 40-50% protein were statistically similar and significantly higher than the other treatments. The larval length was measured after a week. The highest length was recorded in the group fed diet containing 45% dietary protein; whereas, the lowest values were observed the fish fed diets containing 25 and 30% crude protein levels. There were no significant differences in the fish egg amino acid profile (Table 4) and proximate composition (Table 5) among the treatments.

Table 4: Egg amino acid composition (%) of Paradise fish fed different dietary protein levels. Values with the same superscript within the same column are not significantly different (Duncan test, $p \geq 0.01$; n = 3).

Dietary protein (%)	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
25.0	2.2±0.04^{a}	0.8±0.03^{a}	2.2±0.1^{a}	2.5±0.1^{a}	2.6±0.03^{a}	2.6±0.1^{a}	1.4±0.02^{a}	1.64±0.01^{a}	0.9±0.01^{a}	1.8±0.05^{a}
30.0	2.2±0.03^{a}	0.9±0.01^{a}	2.1±0.1^{a}	2.4±0.1^{a}	2.6±0.03^{a}	2.6±0.2^{a}	1.4±0.02^{a}	1.61±0.01^{a,b}	1±0.07^{a}	1.8±0.08^{a}
35.0	2.1±0.02^{a}	0.8±0.02^{a}	2.1±0.1^{a}	2.3±0.2^{a}	2.5±0.07^{b}	2.7±0.3^{a}	1.4±0.01^{a}	1.64±0.01^{a}	1±0.06^{a}	1.8±0.02^{a}
40.0	2.2±0.01^{a}	0.9±0.02^{a}	1.9±0.1^{a}	2.4±0.1^{a}	2.7±0.03^{a}	2.8±0.02^{a}	1.46±0.02^{a}	1.59±0.01^{a}	0.9±0.01^{a}	1.8±0.03^{a}
45.0	2.2±0.01^{a}	0.8±0.02^{a}	2.0±0.1^{a}	2.5±0.1^{a}	2.9±0.04^{a}	3.1±0.1^{a}	1.46±0.03^{a}	1.64±0.02^{a}	0.9±0.01^{a}	1.8±0.04^{a}
50.0	1.9±0.03^{a}	0.8±0.03^{a}	1.9±0.1^{a}	2.3±0.1^{a}	2.9±0.03^{a}	2.9±0.3^{a}	1.44±0.02^{a}	1.54±0.01^{a}	0.9±0.01^{a}	1.8±0.01^{a}

Table 5: Proximate composition (dry matter basis) of eggs in paradise fish fed different dietary protein levels. Values with the same superscript within the same column are not significantly different (Duncan test, $p \geq 0.01$; n = 3).

Dietary protein (%)	Crude Protein (%)	Crude Fat (%)	Ash (%)
25.0	32.9±0.5^{a}	64.7±0.3^{a}	1.9±0.1^{a}
30.0	33.5±0.1^{a}	65.0±0.9^{a}	2.1±0.2^{a}
35.0	33.6±0.4^{a}	63.8±0.7^{a}	1.9±0.3^{a}
40.0	33.7±0.9^{a}	62.9±0.8^{a}	2.2±0.1^{a}
45.0	34.4±0.8^{a}	64.3±0.7^{a}	1.9±0.3^{a}
50.0	33.9±0.3^{a}	64.1±0.2^{a}	2.0±0.2^{a}

Discussion

Dietary protein is an important component of fish feed that directly affects fish growth and reproduction performance. The present study, for the first time, demonstrated optimum dietary protein requirement for paradise fish (Oliva-Teles 2012). Sub-optimal dietary protein levels fail to meet the fish requirement for growth (Siddiqui et al. 1998); this explains the lower growth rate and feed efficiency in the fish fed diets containing 25-35% protein. On the other hand, surplus levels of dietary protein, also, causes growth and health retardation because of increased amino acid catabolism and ammonia production and higher energy expenditure to deaminize excessive amino acids (Dabrowski 1977). This explains decline in the fish growth when fed the diet containing 50% protein, in the present study. In line with the present results, Baishya *et al.* (2012) found that growth performance of snakeskin gourami, *Trichogaster pectoralis*, increase along with elevation in dietary protein levels from 25 to 40 %, and declined afterward. (Mohanta et al. 2013) reported similar results in blue gourami, *Trichogaster trichopterus*, fed diets containing 30, 35, and 40% protein. Regression analysis showed 40 and 45% dietary protein for maximum growth rate of paradise fish, based on SGR and FCR, respectively. However, based on R^2 coefficient, the value 45% seems more reliable; some reproductive traits confirmed this value, too. This level of optimum dietary protein for paradise fish is higher than those reported in snakeskin gourami (40%) (Baishya et al. 2012) and blue gourami (35%) (Mohanta et al. 2013), which may reflect differences in fish species and experimental conditions.

Dietary protein levels and sources have great effects on fish reproductive performance, because certain amino acids at certain amount are needed to produce reproduction-related proteins, such as vitellogenin and gonadotropins (Brooks et al. 1997) . Suboptimal dietary protein provide deficient amount of amino acids needed to produce sufficient number of oocyte with optimal nutrient storage (Singh et al. 2003). Such nutrient deficiencies lead to inferior hatchability, smaller and weaker larvae. Thus, the present results suggest that paradise fish needs 45 % dietary protein to have maximum reproduction efficiency. Similarly, Muchlisin and Hashim (2006) found that highest fecundity and egg diameter in Nile tilapia in fish fed with a diet containing 40% protein, compared to those fed diets containing 30 and 35% protein. However, Afzal Khan et al. (2005) found the best reproduction normative on ruho carp at dietary protein level of 25%, which were superior compared to the fish fed diet containing 200% protein, but showed no difference compared to the fish fed 30-40 % dietary protein. In addition, Chong *et al.* (2004) found that fry productions per mg protein intake of broodstocks were similar in swordtail fed 25-600% dietary protein, which was significantly higher than the fish fed 200% dietary protein. However, 50-600% dietary protein led to maximum

weight gain, relative fecundity and fry production per broodstock.

Fecundity is an important character in reproductive studies of fish and is known to be affected by dietary in broodstock (Izquierdo et al. 2001). The highest fecundity was observed in fish receiving a diet with 45 % crude protein. On the other hand, the lowest fecundity was in the fish fed 50% CP, probably due to the fact that fecundity can vary by the level of protein up to a certain critical level, beyond which additional protein may have no positive effect. The critical CP level may be influenced by age, size and the species involved. Ghaedi *et al*. (2017), reported maximum fecundity in *C. striatus* at 45% CP. In tilapia, swordtail and bagrid catfish, provision of adequate protein resulted in superior fecundity (Abidin et al. 2006; Al Hafedh et al. 1999). When feeding with extremely high or low protein amount, an obvious influence may appear on fecundity may appear. The inferior fecundity in fish fed lower dietary protein, indicates that these protein levels are below the requirement for proper oocyte development in female paradise fish.

Egg size may vary both within a species and between population (Beacham and Murray 1987). Protein value did not alter the egg diameter among treatments because they probably reached the maximum diameter for paradise fish. In contrast Ghaedi *et al.* (2017) reported egg diameter of *C. striatus* was significantly influenced by dietary protein levels. The author concluded that the higher protein intake would lead to larger eggs due to higher protein deposition and nutrient accumulation in the fish egg. Kabir *et al.* (2015) Investigated the effect of different dietary protein levels on pangasius and observed larger eggs in the group of 35 g/ kg CP. The author concluded the differences in egg diameter might have been caused by a difference in metabolic activities rate in the fish liver during the vitellogenesis and nutrient accumulation. There is still a considerable debate on the advantage of producing larger egg in fish (Brooks et al. 1997). Furthermore, studies with tilapia and grass carp showed no relationship between dietary protein and egg size (Gunasekera et al. 1997; Khan et al. 2004).

Although no significant differences were observed among groups in terms of fertilization rate, the highest and lowest hatching rates were in the group of 45 and 25% CP respectively. The fertilization rate is affected by the endocrine status of gamete. The ability of egg and sperm to be blended and fertilized, is related to gamete quality as well as nutritional and environmental status (Karga and Mandal 2017). However, the husbandry practices, the physiochemical condition of the water, temperature, and embryonic development progress may influence the hatching rate. Therefore, it is concluded that the fertilization rate and hatching rate are altered by non-controllable biological, nutritional and environmental condition. The similar result reported by (Gonzales and John 2012; Karga and Mandal 2017) on zebrafish, on bighead carp (Santiago et

al. 1991) and (Ghaedi et al. 2017) on snakehead.

In the present study, there was no change in egg proximate composition and amino acid profile at different dietary protein levels, which was similar to the results found in Nile tilapia (Gunasekera et al. 1995). Therefore, it seems that inferior reproductive performance of the fish fed non-optimal dietary protein was not related to protein and amino acid supply to the eggs. This might be due to lower health condition of the broodstocks fed non-optimal dietary protein levels, which might cause nutritional stress (Abdel-Tawwab 2012). It is well-known that exposure of broodstock to stressful conditions causes reproduction retardation in fish (Barton 2002; Mazandarani et al. 2015). This topic needs further evaluation in paradise fish.

Conclusion

In conclusion, dietary protein level of 45% is necessary for maximum growth and reproduction performance of paradise fish. Higher level of dietary protein (50%) brings no further improvement in fish growth and reproduction, but has negative effects. Moreover, dietary 40% protein cause growth performance similar to 45% dietary protein, but fails to induce similar reproduction performance.

Acknowledgment

This project was recorded at the Registration System for Research Projects with the registration code of 4-12-12-93115 and financially supported by the Iranian Fisheries Science Research Institute. The authors are grateful to Dr. Hassanpour for his statistical counseling. Thanks goes to the manager of the ornamental fish breeding center, Mr Jamshidi for providing necessary facilities for the experiment and Dr. Hoseini for all his kind supports.

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