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Growth Rate, Proximate Composition and Fatty Acid Profile of Juvenile kutum *Rutilus frisii kutum* Under Light/Dark Cycles

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Abstract

This study investigated the growth rate, proximate composition, and fatty acid profile in juvenile of kutum *Rutilus frisii kutum* in different dark-light cycles. Fish were stocked randomly in eighteen 2000-L fiberglass tanks and received six photoperiod regimes: natural photoperiod, 24L (light):0D (day), 16L:8D, 12L:12D, 8L:16D and 0L:24D. Application of continuous dark (0L:24D) and an intermediate photoperiod (12L:12D) resulted in the highest specific growth rate (SGR) as well as lowest feed conversion ratio (FCR) ($P < 0.05$) in kutum. The quantity of crude protein and ash of kutum subjected in continuous dark (0L:24D), exhibited higher rate than other photoperiod regimes. The proportion of DHA (docosahexaenoic acid) +EPA (eicosapentaenoic acid) content ranged from 3.20% in natural photoperiod to 3.52% in continuous dark, n-3/n-6 and PUFA/SFA fatty acids ratio slightly ranged from 0.15-0.18, and 0.99-1.08 in all photoperiod regimes, respectively.

Keywords: Dark-light cycle · Kutum, *Rutilus frisii kutum* · Proximate composition · Fatty acid · Growth performance.

1. Introduction

Environmental and nutritional factors notably influence fish growth. In addition to temperature, light/dark cycle is an important factor that affects organisms including fish. Alteration of artificial photoperiod can induce physiological and immunological changes in fishes (Valenzuela *et al.*, 2008). These changes can be recognized through hormonal variation and alteration of blood parameters, such as cell number and volume (Zarejabad *et al.*, 2009). Taylor *et al.* (2006) believed that artificial light regimes have the ability to increase growth rates up to 25% in farmed rainbow trout *Oncorhynchus mykiss*.

The effects of photoperiod on growth rate and other variables have been studied in various species (Krakenes *et al.*, 1991; Imsland *et al.*, 1995; Davis *et al.*, 1999; Jonassen et al., 2000; Kissil et al., 2001; Petit et al., 2003; Norberg et al., 2004; Bayarri *et al.*, 2004; Taylor *et al.*, 2006; Valenzuela *et al.*, 2006; Ruchin, 2007; Bani *et al.*, 2009; Ghomi *et al.*, 2010a; Ghomi *et al.*, 2010b).

kutum *Rutilus frisii kutum* is a commercially important fish available in the southern waters of the Caspian Sea and some lakes of Turkey with high market acceptance. A decrease in water levels in the Caspian Sea from the 1950s has led to a drastic decline in the stocks (Afraei Bandpei, 2010). Artificial propagations of kutum are carried out every year to produce fingerlings to be released into the rivers in the Caspian Sea and the amount of restocking and catching were 187.1 million individual fish and 14835 ton in 2008, respectively (Abdolhay *et al.*, 2010).

There are many studies on the effect of photoperiod on teleosts; however, physiological responses of kutum juvenile in captivity condition to different photoperiod regimes are not well known. Thus, this study aims at measuring the growth rate, proximate composition, and fatty acid profile in juvenile of kutum in different dark-light cycle.

2. Materials and Methods

2.1. Experimental materials and fish

Kutum juveniles (1.39 ± 0.3 g) were provided from a local hatchery (Rajaee Fish Farm Center, Sari, Iran) and fed with a grinded basal diet of carp (32% protein, 10% fat, 15% ash, 5% fiber, and 11% moisture) (Isfahan Mokamel, Iran) 10% of body weight. The juveniles acclimatized in the two 2000-L fiberglass storage tanks and fed basal diet (4 times daily) for 1 week prior to the commencement of the experiment. Acclimated fish were distributed randomly in eighteen 2000-L fiberglass tanks (with three replicates for each treatment) each of which was filled each with 1000 L of well water. Fish received six photoperiod regimes: natural photoperiod, 24L (L = light):0D (D = dark), 16L:8D, 12L:12D, 8L:16D and 0L:24D. All tanks were covered by black nylon sheets. Light cycles were provided by incandescent lamps (20 W, Nama Noor, Tehran, Iran), which were installed 1 m above water surface, equipped with digital timers (Everflourish Electrical Co, China).

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During the experimental period, aeration was continuously supplied and different physico-chemical parameters of the rearing water such as temperature, dissolved oxygen, and pH were routinely monitored. The pH and temperature varied in the range of 7.9-8.3 and 17-24 °C, respectively. Water flow was 5 l/min and the dissolved oxygen content was more than 7.0 mg/l. The fish were stocked at 200/tank and cultured for 60 days.

Sampling was carried out at the beginning of study and on days 30 and 60 after rearing by removing the 50 juveniles of each treatment at each time. The specific growth rate and survival were measured by counting the number of juveniles at each sampling time, measuring the weight to nearest 0.01 g. The SGR and survival rate were calculated as follows: SGR (%g/day)= 100× ($\ln W_t$ - $\ln W_o$)/t; and survival (%)= (N_t-N_o) ×100, where W_t and W_o are the weight of the juveniles at day t and at the beginning of the experiment, respectively. N_t and N_o are the number of juveniles at the end (t) and beginning (o) of the study. Feed conversion ratio (FCR) was calculated as FCR= Fc/ΔW, where Fc is the total dry food consumed by the fish and ΔW is the total weight gained (g).

2.2. Proximate composition

Moisture content was determined by drying the 5 g minced fish at 105 °C until a constant weight was obtained (AOAC, 2005). Lipid was extracted according to Kinsella ***et al.*** (1977). Fifty g of whole body were homogenized in a warring blender (32BL79, New Hartford, Connecticut, USA) for 2 min with a mixture of 50 ml chloroform and 100 ml methanol. Then 50 ml of chloroform were added and further homogenized. Finally, 50 ml of distilled water were added to the mixture and blended for 30 sec. The homogenate was filtered through a whatman No. 4 filter paper into a decantor (Witeg, Germany). The lower fraction was then collected and filtered. It was then transferred to a rotary evaporator (Rotavapor R-114, BÜCHI, Switzerland) for solvent evaporation. Lipid content was expressed as gram per 100 g wet whole body. Crude protein content was calculated by converting the nitrogen content determined by Kjeldahl method (6.2×N) (AOAC, 2005). Ash content was determined gravimetrically in a furnace by heating at 550°C to constant weight (AOAC, 2005).

2.3. Fatty acid profile

Fatty acid methyl ester (FAME) was prepared following the method of Timms (1978). Lipid samples (0.2 g) were weighed and diluted with 4 ml of hexane followed by the addition of 0.2 ml of sodium methoxide in a sealed tube. The mixture was then shaken using a vortex for 1 s and left for about 30 min until it separated into two phases. The top layer, FAME was then taken for analysis by using Trace GC (Thermo Finnigan, Italy). The GC conditions were as follows: capillary column (Bpx-70 60 m x 0.25 mm i. d. × 0.25µm), the split ratio was 80:1. Injection port temperature was 250 °C; flame ionization detector temperature was 270 °C. Oven temperature was set at 194 ºC for 90 minutes. Flow rate of carrier gas (helium) was 1 mL/min and the make up gas was N_2 (30 ml/min). The sample size injected for each analysis was 1 µL. Samples were manually injected into the GC port. Compounds were identified in comparison to retention times of known standard (Vingering and Ledoux, 2009).

2.4. Data analysis

The ANOVA assumptions of normality, homogeneity of variance (Little and Hills 1978) were examined using the Shapiro-Wilk and the Levene tests, respectively. The differences between groups were analyzed by using one-way ANOVA and Duncan's multiple range tests at $P <$ 0.05.

3. Results and Discussion

Mean weight of fish during experimental duration under different regimes of photoperiod has been presented in Figure 1. Continuous dark (0L:24D) gained the greatest final weight of fish among all photoperiod regimes ($P <$ 0.05). According to Figure 2, application of continuous dark (0L:24D) and an intermediate photoperiod (12L:12D) resulted in the highest specific growth rate (SGR) as well as the lowest feed conversion ratio (FCR) ($P < 0.05$) in kutum. The treatment 5 (8L:16D) insignificantly exhibited the lowest mortality among all photoperiod treatments. A significant decrease in SGR can be seen in the natural photoperiod (Fig. 2). These results in the better growth rate of an intermediate photoperiod (12L:12D) were in accordance with some species. Pavlidis ***et al.*** (1999) pointed out that the highest growth rate and food utilization efficiency in *Pagrus pagrus* were recorded under 12 h photoperiod, while the negative growth was observed in the dark. Also, the highest weight gain for European eel *Anguilla anguilla* observed under 12 h light/dark regime and decreased food coefficient in the dark (Meske, 1982). Similarly, the maximum SGR and minimum FCR have been observed by an intermediate light/dark cycle (12L:12D) ($P < 0.05$) in beluga sturgeon *Huso huso* (Ghomi ***et al.***, 2010a). The highest survival rate (89%) in juvenile bluga sturgeon *Huso huso* was observed in the 12L:12D period, but differences in growth were insignificant for the photoperiod regimes (Bani ***et al.***, 2009). The maximum growth rate of juvenile Siberian sturgeon, *Acipenser baerii*, was in 12, 16, and 24 h photoperiod (Ruchin, 2007). Thus, exerting either continuous dark (0L:24D) or an intermediate photoperiod (12L:12D) may cause a better condition for growth of kutum juvenile.

In contrast, Ghomi ***et al.*** (2010a) pointed out that the continuous dark (0L:24D) and the continuous light (24L:0D) significantly ($P < 0.05$) reduced the final weight of fish in beluga sturgeon *Huso huso* and are not compatible with the physiological condition of this species. A physiological deterioration can be recognized in carp yearlings in the dark as well as their better physiological state in the 12D:12L, 16D:8L, and 0D:24L variants (Ruchin, 2006). Similarly, Semenkova and Trenkler (1993) reported that mean weight of 4-month old beluga sturgeon *Huso huso* exposed to a 24 h photoperiod was 15% lower compared to a 16 h photoperiod. The highest growth rate of *Acipenser nudiventris* was observed under a 24 h photoperiod (Ponomarenko ***et al.***, 1992). Juvenile rainbow trout *Oncorhynchus mykiss* exposed to 18L:6D grew to a significantly heavier mean weight than the other treatments (Taylor ***et al.***, 2005).

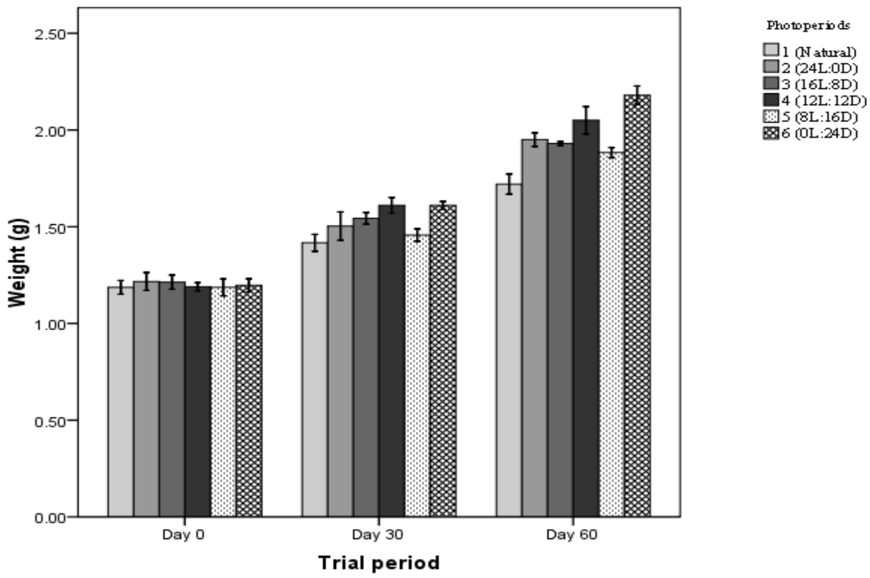

Figure. 1. Mean weight of kutum during 60 days trial under different regimes of photoperiod

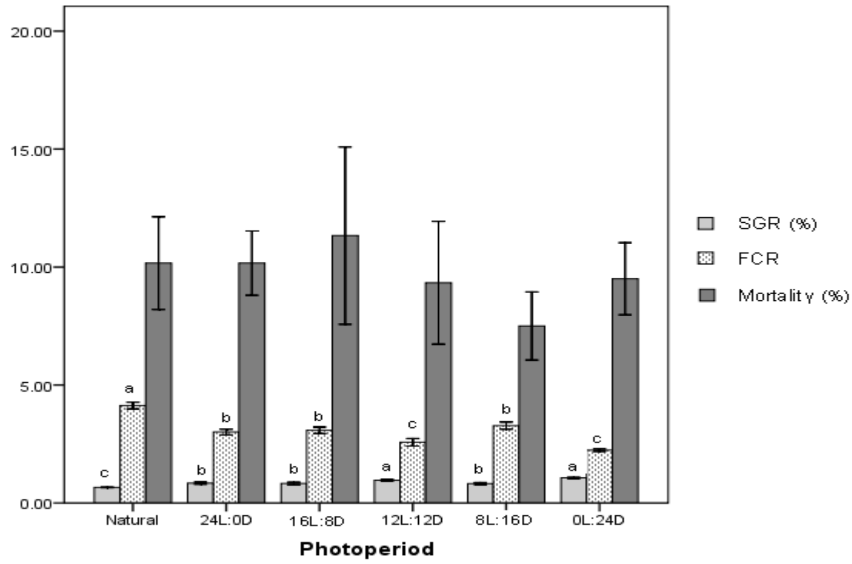

Figure. 2. SGR, FCR and mortality of fish under different regimes of photoperiod. Mean values with the same letter for same labeled columns are not significantly different ($P > 0.05$).

Proximate composition of fish is shown in Table 1. The crude fat, crude protein, ash and moisture content of kutum under various photoperiods were significantly different from each other ($P < 0.05$). This is the first report for the investigation of the photoperiod effect on proximate composition and fatty acid profile in kutum. The highest crude fat content in this study for kutum was 5.01% for treatment 3 (16L:8D). Mean protein content of kutum was in the range of 14.44% (for treatment 3 (16L:8D)) and 15.38% (for treatment 6 (0L:24D)) (Table 1). The maximum amount of moisture (75.26%) and ash (2.19%) was gained for treatment 4 (12L:12D) and 6 (0L:24D), respectively. Consequently, the quantity of crude protein and ash of kutum subjected in continuous dark (0L:24D) exhibited a higher rate than other photoperiod regimes. The proximate content values for the kutum in this study were mostly in the range of previous studies. Pirestani *et al.* (2009) and Keyvan et al. (2008) reported the amount of fat, protein, ash and moisture content in kutum (6.70%, 21.40%, 1.30%, 72.40%) and (3.21%, 21.80%, 1.29%, 75.90%), respectively. Kutum belongs to low-fat fish species, and by increasing the fat content, the rate of moisture was reduced (r = -0.656, $P < 0.01$) (Table 1). This fact was in agreement with Osman *et al.* (2001) which pointed out that low-fat fish species have higher water content. The increase in the moisture content was found to be associated with increased protein and ash contents in fish in this experiment (Table 1).

The fatty acid composition of fish under different photoperiod is summarized in Table 2. The fatty acid composition of kutum ranged from 0.37% for C22:4n-6 to 17.30% for C18:1n-9. A good indicator for comparing nutritional values of oils is the ratio of n-3/n-6. In the present study, n-3/n-6 ratio ranged from 0.15 to 0.18 (Table 2) which was not close to the range (0.5-3.80) given by Henderson and Tocher (1987) for freshwater species. Freshwater fish are generally characterized by high levels of n-6 PUFA, especially linoleic acid (18:2n-6) and arachidonic acid (20:4n-6) (Ozogul *et al.*, 2007). The same result was observed in the present study for linoleic acid (18:2n-6) and arachidonic acid (20:4n-6) up to the rate of 16.48% and 4.35%, respectively. Since freshwater fish contain lower proportions of long-chain n-3 PUFA than marine fish (Rahman *et al.* 1995), the ratio of total n-3 to n-6 fatty acids is much higher for marine fish than freshwater fish, varying from 5 to 10 or more (Ozogul *et al.*, 2007).

Table 1. Proximate composition (g/100g wet weight) of kutum subjected to different photoperiod regimes, and intercorrelation among proximate parameters

Photoperiod regimes	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)
1 (Natural)	73.51±0.49[c1]	15.16±0.10[ab]	4.36±0.06[c]	1.77±0.02[c]
2 (24L:0D)	72.75±0.05[d]	14.75±0.26[c]	4.77±0.02[b]	1.66±0.00[c]
3 (16L:8D)	73.05±0.03[d]	14.44±0.05[d]	5.01±0.09[a]	1.65±0.00[c]
4 (12L:12D)	75.26±0.35[a]	15.22±0.10[a]	4.33±0.04[c]	1.96±0.06[b]
5 (8L:16D)	74.19±0.02[b]	14.94±0.15[bc]	4.62±0.11[b]	1.71±0.20[c]
6 (0L:24D)	74.29±0.10[b]	15.38±0.02[a]	4.37±0.12[c]	2.19±0.03[a]
Intercorrelation				
Moisture	1	0.645[**2]	-0.656[**]	0.631[**]
Crude protein		1	-0.848[**]	0.677[**]
Crude fat			1	-0.688[**]
Ash				1

[1] Mean values with the same letter for each column are not significantly different ($P > 0.05$).
[2] Significant at $P < 0.01$.

Table 2. Fatty acid composition (% total fatty acids by peak area) of kutum subjected to different photoperiod regimes

	Photoperiod regimes					
Fatty acid	Natural	24L:0D	16L:8D	12L:12D	8L:16D	0L:24D
C14:0	3.23	4.44	3.80	3.65	4.12	3.98
C16:0	15.75	16.10	15.08	16.30	15.60	16.13
C18:0	8.35	8.46	7.17	7.90	7.68	7.83
Σ SFA[*]	27.73	29.00	26.05	27.85	27.40	27.94
C16:1n-7	1.77	1.85	1.63	1.68	1.60	1.56
C18:1n-7	7.04	8.35	9.52	9.03	7.30	8.10
C18:1n-9	15.10	16.20	14.25	15.10	17.30	16.15
C20:1n-9	4.75	4.63	4.84	5.07	4.81	5.17
Σ MUFA [*]	28.66	31.03	30.24	30.88	31.01	30.98
C18:2n-6	15.70	16.35	15.84	16.48	16.36	16.10
C20:2n-6	3.65	3.72	3.83	3.80	3.89	2.95
C20:4n-6	4.17	4.23	4.04	3.86	4.11	4.35
C20:5n-3 (EPA)	1.05	1.33	1.41	1.38	1.47	1.39
C22:4n-6	0.39	0.38	0.40	0.45	0.36	0.37
C22:5n-3	0.59	0.65	0.80	0.71	0.42	0.82
C22:6n-3 (DHA)	2.15	2.07	1.91	1.95	2.04	2.13
Σ PUFA [*]	27.70	28.73	28.23	28.63	28.65	28.11
n-3/n-6	0.15	0.16	0.17	0.16	0.15	0.18
EPA+DHA	3.20	3.40	3.32	3.33	3.51	3.52
PUFA/SFA	0.99	0.99	1.08	1.02	1.04	1.00

[*] SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids.

Fish lipids have useful polyunsaturated fatty acids, particularly n-3 fatty acids, which play an important role in human health promotion. Mozaffarian *et al.* (2005) believed that DHA and EPA had been reported to have preventive effects on human coronary artery disease. The proportion of DHA and EPA content ranged from 3.20% in natural photoperiod to 3.52% in continuous dark (Table 2). The amount of the DHA+EPA content in our study was lower than that of some other species. The low levels of DHA and EPA in the kutum juvenile farmed in freshwater, in present study, were not surprising when compared with their seawater un-growing size (11.69%, reported by Pirestani *et al.*, 2010) because seawater fish obtain higher omega-3 fatty acid from more diversity of seafood. The fatty acid composition of different individual fish of the same species can vary because of diet, location, gender, and environmental conditions (Gruger, 1967). EPA+DHA content was found to be 7.88% for *Acipenser oxyrinchus desotoi* (Chen *et al.* 1995), 15.71% for cultured sturgeon (*A. baerii*, *A. naccarii,* and *A. transmontanus*; Badiani *et al.*, 1996). EPA+DHA content in *Clarias gariepinus* and *Tilapia zillii* was 4.2% and 4%, respectively (Osibona *et al.*, 2009). Ozogul *et al.* (2007) reported EPA+DHA mean content was various in *Clarias gariepinus* (8.82%), *Cyprinus carpio* (14.07%), *Siluris glanis* (17.56%), *Tinca tinca* (25.51%), *Rutilus frisii Kutum* (23.5%), *Sander lucioperca* (28.39%). Since the minimum value of the PUFA/SFA ratio recommended is 0.45 (HMSO 1994), in present study, PUFA/SFA fatty acids ratio was slightly ranged from 0.99 to 1.08 in all photoperiod regimes (Table 2).

As a conclusion, artificial regulations of photoperiod regimes may cause a better condition for the growth of kutum juvenile, significantly affecting proximate composition, but they did not affect fatty acid, comparing indicators like PUFA/SFA, n-3/n-6, and EPA+DHA content in kutum.

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