

## Effects of different feed restriction periods on the growth and fatty acid compositions in juvenile rainbow trout (*Oncorhynchus mykiss*)

Baki B.<sup>1\*</sup>; Kaya Öztürk D.<sup>1</sup>; Kerim M.<sup>1</sup>; Eyuboglu B.<sup>2</sup>

Received: August 2016

Accepted: October 2018

### Abstract

The aim of this study was to determine the effect of different feed restriction periods on the growth performance as well as the biochemical and fatty acid compositions of fillet in rainbow trout (*Oncorhynchus mykiss*). Fish with an average initial weight of  $64.80 \pm 7.41$ g were used in the study. The trial lasted 60 days and 4 different feeding diets were alternately applied to the fish. The control group (C) was fed continuously throughout the trial while the other groups were fed 2 days starvation 1 day<sup>-1</sup> feeding (2D), 4 days starvation 1 day<sup>-1</sup> feeding (4D), and 6 days starvation 1 day<sup>-1</sup> feeding (6D). The average weight of the fish at the end of the trial was  $219.78 \pm 31.32$ g (C),  $168.41 \pm 21.44$ g (2D),  $116.60 \pm 12.28$ g (4D), and  $87.64 \pm 12.99$ g (6D), respectively. The fillet protein values were determined as  $20.85 \pm 0.69$  (C),  $19.82 \pm 0.68$  (2D),  $18.19 \pm 0.79$  (4D), and  $18.42 \pm 1.21$  (6D), respectively. The lipid values were  $6.18 \pm 0.40$  (C),  $3.35 \pm 0.41$  (2D),  $2.26 \pm 1.63$  (4D), and  $1.94 \pm 0.63$  (6D), respectively. The lipid lean<sup>-1</sup> body mass values were  $0.27 \pm 0.05$  (C),  $0.16 \pm 0.03$  (2D),  $0.08 \pm 0.05$  (4D), and  $0.11 \pm 0.03$  (6D), respectively. Regarding the analyses conducted on fish muscle tissues, the differences between the control group and feed restriction groups were statistically significant in terms of saturated fatty acids, monounsaturated fatty acid, polyunsaturated fatty acid, Omega-3, Omega-6, and Omega-9 values. In conclusion, it has been determined that the different feed restriction periods in the feeding of rainbow trout had an effect on the duration of reaching the marketable weight, feed conversion rates, meat yield, fillet protein, and fat ratio values and increased reaction to feed.

**Keywords:** *Oncorhynchus mykiss*, Feed restriction, Growth, Feed consumption, Biochemical composition

1- Department of Aquaculture, Faculty of Fisheries, Sinop University, Sinop, Turkey.

2- School of fisheries and aquaculture, Higher Vocational School, Sinop University, Sinop, Turkey.

\*Corresponding author's Email: [bbaki@sinop.edu.tr](mailto:bbaki@sinop.edu.tr)

## Introduction

Nutrition is the most important activity that determines all of the vital features of living things and has a great impact on growth and costs. Therefore, feeding is of importance in sustainable aquaculture systems. The purpose of fish feeding is to reach the yield weight at the most appropriate time, in addition to reduce the feed and other costs as well as to determine the economically sustainable and environmentally friendly feeding protocols. In this manner, there are studies conducted on the most efficient feeding models that effect feed conversion and fish growth (Foss *et al.*, 2009; Føre *et al.*, 2016). In recent studies, researchers have focused on starvation periods and limited feeding regimes and their effects on growth performances (Chatakondi and Yant, 2001; Foss and Imsland, 2002; Heide *et al.*, 2006; Eroldoğan *et al.*, 2008; Taşbozan *et al.*, 2014).

In their natural environment, fish are exposed to feed deprivation in certain periods of the year during behaviors including escaping from predators, thermoregulation, and reproduction (McCue, 2010). In addition, they can be exposed to short-term or long-term starvation periods in aquaculture conditions in certain times of the year due to some environmental factors (negative weather and sea conditions) and production methods (Takagi, 2001; Perez-Jimenez *et al.*, 2007; Eroldoğan *et al.*, 2008).

The aim of this study was to determine the effect of different starvation periods on the growth performance as well as the biochemical and fatty acid compositions of

fillet in rainbow trout (*Oncorhynchus mykiss*).

## Materials and methods

### *Experimental organisms, culture system and feed regimes*

Fish with an average initial weight of  $64.80 \pm 7.41$  g and an average length of  $19.60 \pm 1.00$  cm were used in the study. Fish were received from Kızılırmak Aquaculture (Samsun-Turkey). The salmon were transferred from the hatchery (freshwater) to the Sinop University Faculty of Fisheries, Aquatic Research Building. The fish were randomly distributed (360) into 12 experimental tanks (300 L), each tank with 30 fish. Water inflow was adjusted to  $4 \text{ L min}^{-1}$  and supplemental aeration was provided via air stone diffusers.

The fish were acclimated on the control diet for one week prior to initial sampling. The study was conducted in 3 repetitions ( $p=0.940$ ) and four different feeding regimes were for 60 days. The control group (C) was fed continuously throughout the trial while the other groups were fed 2 days starvation/1 day feeding (2D), 4 days starvation/1 day feeding (4D), and 6 days starvation/1 day feeding (6D). The fish were fed two times a day to satiation. Commercial trout feed (Black Sea Feed/Sinop, Turkey) with 45/20 (%) protein/fat ratio were used for fish feeding (Table 1).

**Table 1: Biochemical composition of the diets used in the experiment.**

Biochemical Composition	
Moisture % (max)	10
Crude Protein % (min)	45
Digestible Protein (%)	40.8
Crude Lipid % (min)	20
Crude Ash % (max)	10
Crude Cellulose % (max)	3
Gross Energy (Kcal kg <sup>-1</sup> ) (min)	4801
Digestible Energy (Kcal kg <sup>-1</sup> ) (min)	4379
Metabolic Energy (Kcal kg <sup>-1</sup> ) (min)	3909
Omega-3 (g kg <sup>-1</sup> ) (min.)	42
Omega-6 (g kg <sup>-1</sup> )	12
Omega-3/ Omega-6	3.5
Calcium % (min-max)	1-3

This study was conducted in compliance with the rules for animal experiments for scientific purposes and permission was given by the Sinop University Animal Experiments Local Ethics Committee with the permission No. 2014/09 on April 16<sup>th</sup> 2014.

#### Water quality

Water quality parameters were monitored twice a day (09<sup>00</sup> and 16<sup>00</sup> hours). The measured average water temperature was 17.03±0.97°C (15.9-19.3), the average oxygen content was 6.54±0.65 (5.69-7.79) mg L<sup>-1</sup>, and the average pH value was 7.88±0.48 (6.6-8.2).

#### Growth performance

The growth parameters of the fish and biochemical composition of the fillet were determined by taking random samples from each group at the baseline, every 15 days, and at the end of the trial. Growth performance (Specific Growth Rate, Daily Growth Coefficient, Feeding Day Growth Coefficient, Feed Conversion Rate, Feed Consumption Rate, Protein Efficiency Rate) (Hoşsu *et al.*, 2005; Turchini *et al.*,

2011); viscerosomatic index, hepatosomatic index, carcass yield, and condition factor values were calculated (Skalli and Robin, 2004; Cui *et al.*, 2006; Sevgili, 2007).

Specific Growth Rate (SGR), % =  

$$[(\ln \text{ Final weight, g} - \ln \text{ Initial weight, g}) / \text{Day}] \times 100$$

Daily Growth Coefficient =  

$$(\text{Final weight, g} - \text{Initial weight, g}) / \text{The number of trial days}$$

Feeding Day Growth Coefficient =  

$$(\text{Final weight, g} - \text{Initial weight, g}) / \text{The number of feedig days}$$

Feed Conversion Rate (FCR) =  

$$\text{Total consumed amount of feed, g} / \text{Total weight gain, g}$$

Feed Consumption Rate =  

$$(\text{Daily individual consumed amount of feed, g} / \text{Average fish weight, g}) \times 100$$

Protein Efficiency Rate =  

$$(\text{Live weight gains, g} / \text{Protein intake, g}) \times 100$$

VSI (%) =  

$$(\text{Vicera weight, g} / \text{Total body weight, g}) \times 100$$

HSI (%) =  

$$(\text{Liver weight, g} / \text{Total body weight, g}) \times 100$$

Carcass Yield (%) =  

$$(\text{Edible fillet weight, g} / \text{Total body weight, g}) \times 100$$

Condition Factor =  $(W/L^3) \times 100$

*Proximate composition and fatty acid analysis*

The fillet crude protein (%) analysis was carried out according to Weende analysis method, crude fat (%) analysis was performed according to Acid Hydrolysis Soxtec System Method, and moisture (%) analysis was carried out according to drying method Association of Official Analytical Chemists (AOAC, 2000). The fillets were stored at -20°C until the time of biochemical analysis.

Lipid/Lean Body Mass was calculated according to Sevgili *et al.* (2013) and the method of calculation was indicated below.

$$\text{Lipid/Lean Body Mass (L/LBM)} = \frac{\text{Whole body lipid, g}}{\text{Whole body protein, g} + \text{Whole body ash, g}}$$

Fatty acids analysis was performed according to the IUPAC gas chromatography method (Firestone and Horwitz, 1979) at TUBITAK Marmara Research Center (MAM) Food Institute.

The fish were stored at -80°C freezer until analysis before being transferred.

*Statistical methods*

The data obtained from the analyses were statistically analyzed with one-way ANOVA using the SPSS version 21 statistics software. The differences between the values were compared with Tukey's multiple comparison tests at the  $p < 0.05$  level of significance. Significance test of Between EPA-DHA and fasting period of trial groups was carried out with correlation analysis.

**Results**

It was determined that the final weight, specific growth rate, and daily growth rate values of the starvation groups at the end of the study were lower than those of the control group, and the starvation period had an effect on the growth (Table 2, Fig. 1).

**Table 2: Growth parameters of rainbow trout fish.**

Parameters	Control	2D	4D	6D	p values
Initial Weight (g)	64.77±7.40 <sup>a</sup>	64.87±7.33 <sup>a</sup>	64.77±7.59 <sup>a</sup>	64.80±7.31 <sup>a</sup>	0.940
Final Weight (g)	219.78±31.32 <sup>d</sup>	168.41±21.44 <sup>c</sup>	116.60±12.28 <sup>b</sup>	87.64±12.99 <sup>a</sup>	0.001
SGR (%)	2.18±0.52 <sup>c</sup>	1.70±0.65 <sup>b</sup>	1.05±0.64 <sup>ab</sup>	0.54±0.22 <sup>a</sup>	0.004
Daily Growth Coefficient	2.87±0.04 <sup>c</sup>	1.90±0.25 <sup>b</sup>	0.77±0.10 <sup>a</sup>	0.42±0.05 <sup>a</sup>	0.001
Feeding Day Growth Coefficient	2.87±0.04 <sup>a</sup>	5.71±0.75 <sup>b</sup>	3.48±0.92 <sup>a</sup>	3.73±0.46 <sup>a</sup>	0.011
FCR	1.00±0.02 <sup>a</sup>	0.99±0.30 <sup>a</sup>	1.13±0.43 <sup>b</sup>	1.77±0.99 <sup>c</sup>	0.230
Feed Consumption Rate	0.61±0.21 <sup>a</sup>	1.26±0.16 <sup>b</sup>	1.31±0.33 <sup>b</sup>	1.31±0.25 <sup>b</sup>	0.004
PER	2.22±0.03 <sup>b</sup>	2.39±0.59 <sup>b</sup>	1.69±0.92 <sup>a</sup>	1.50±0.47 <sup>a</sup>	0.019
VSI (%)	12.50±0.99 <sup>b</sup>	12.23±1.22 <sup>b</sup>	13.01±3.74 <sup>c</sup>	10.58±0.92 <sup>a</sup>	0.001
HSI (%)	1.71±0.26 <sup>a</sup>	1.72±0.30 <sup>a</sup>	1.66±0.44 <sup>a</sup>	1.46±0.16 <sup>a</sup>	0.315
CY (%)	53.93±1.90 <sup>b</sup>	49.96±2.56 <sup>ab</sup>	48.27±2.75 <sup>a</sup>	47.92±1.66 <sup>a</sup>	0.003
CF (%)	1.40±0.11 <sup>b</sup>	1.38±0.10 <sup>b</sup>	1.25±0.16 <sup>a</sup>	1.11±0.07 <sup>a</sup>	0.001
Survival Rate (%)	98.33±2.36	93.33±4.71	90.00±9.43	91.67±7.07	-

Values in the same row with different superscripts are significantly different ( $p < 0.05$ )

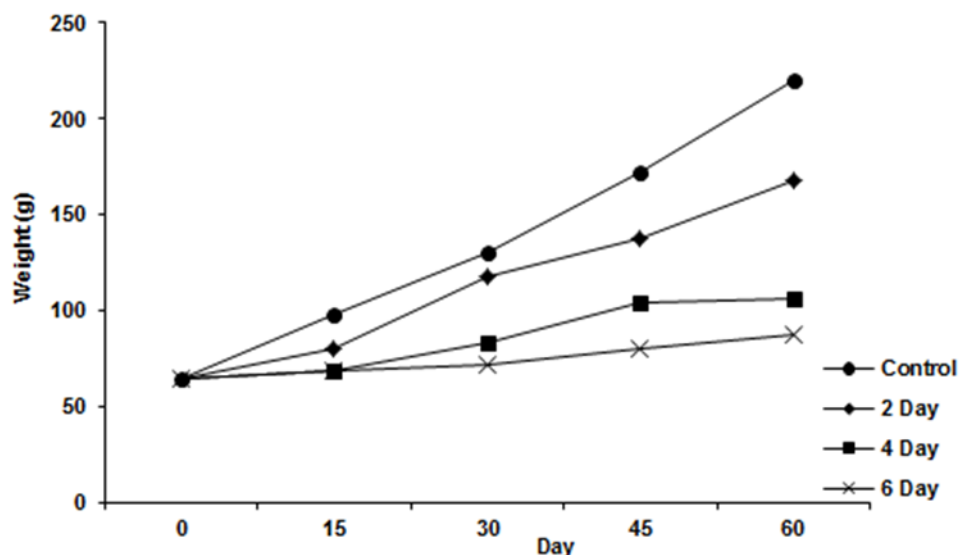


Figure 1: The average fish weights during the trial (g).

The average weight values of the fish were  $219.78 \pm 31.32$  g (165-305 g) in the Control,  $168.41 \pm 21.44$  g (128-224 g) in 2D,  $116.60 \pm 12.28$  g (75-126 g) in 4D, and  $87.64 \pm 12.99$  g (53-116 g) in 6D. The specific growth rate (%) values were  $2.18 \pm 0.52$ ,  $1.70 \pm 0.65$ ,  $1.05 \pm 0.64$ ,  $0.54 \pm 0.22$ , respectively ( $p=0.04$ ). In terms of the feed conversion ratio, the best value was detected in the control group ( $1.00 \pm 0.02$ ) and 2D ( $0.99 \pm 0.30$ ) group ( $p=0.230$ ), while the lowest value was in 6D group ( $1.77 \pm 0.99$ ). In terms of the protein efficacy ratio, it was detected that the best group was 2D ( $p>0.05$ ), and the

differences between this group and other groups were not significant ( $p<0.05$ ).

In terms of VSI, CY, and CF values, the difference between the control group and 2D group was not significant ( $p>0.05$ ), whereas the differences between the other groups were significant ( $p<0.05$ ). In terms of HSI (%) values, the differences between all the groups were not significant ( $p=0.315$ ).

During the feeding days, it was detected that the average feed consumption increased parallel to the starvation period and differences among control group and other groups were significant ( $p=0.04$ ) (Figs. 1, 2).

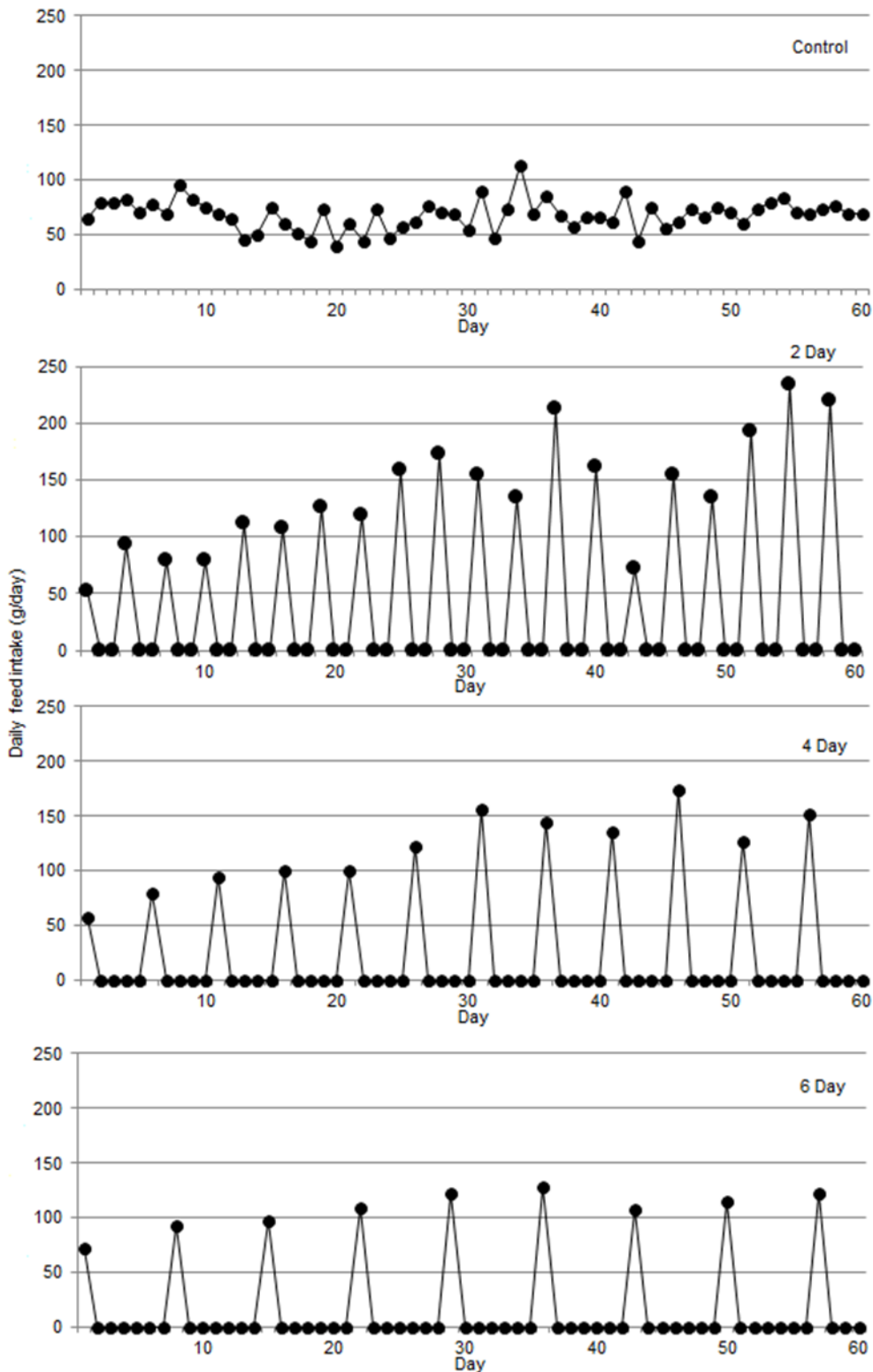


Figure 2: Changes in the total daily feed intake ( $\text{g day}^{-1}$ ).

The fillet biochemical composition values were shown in Table 3 and the fatty acids composition was shown in Table 4. The difference between 4D and 6D groups, and the difference between the control group

and 2D group was significant in terms of the fillet protein values ( $p=0.003$ ). The differences between the control group and starvation groups in terms of the lipid values were significant ( $p<0.05$ ). The dry

matter values in the starvation groups decreased significantly ( $p < 0.05$ ) compared to those of the control group. In terms of the L/LBM values, which indicate fillet fat content values, the differences between the

control group and starvation groups were significant ( $p < 0.05$ ). In the study, the body lipid contents of the starvation groups decreased as the water contents increased.

**Table 3: Body composition (%) values at beginning and the end of the trial\***

	Initial	Final				p values
		C	2D	4D	6D	
Protein	17.31±0.49	20.85±1.06 <sup>b</sup>	19.82±0.90 <sup>b</sup>	18.19±1.08 <sup>a</sup>	18.42±1.00 <sup>a</sup>	0.003
Lipid	2.34±0.79	6.18±0.65 <sup>b</sup>	3.35±0.71 <sup>a</sup>	2.26±0.79 <sup>a</sup>	1.94±0.38 <sup>a</sup>	0.001
Ash	1.30±0.11	1.40±0.06 <sup>a</sup>	1.10±0.22 <sup>a</sup>	1.08±0.22 <sup>a</sup>	1.38±0.08 <sup>a</sup>	0.050
Dry Matter	21.35±0.59	28.59±2.15 <sup>c</sup>	26.15±1.43 <sup>b</sup>	22.36±0.88 <sup>a</sup>	22.83±1.03 <sup>a</sup>	0.001
L/LBM	0.15±0.03	0.27±0.05 <sup>c</sup>	0.16±0.03 <sup>b</sup>	0.08±0.05 <sup>a</sup>	0.11±0.03 <sup>a</sup>	0.001

Values in the same row with different superscripts are significantly different ( $p < 0.05$ )

\*Biochemical analysis were performed on wet weight

**Table 4: Fillets fatty acid compositions (%) at the beginning and end of the trial.**

Fatty Acid	Initial	Final				p values
		C	2D	4D	6D	
C12:0	0.04±0.01 <sup>a</sup>	0.04±0.01 <sup>a</sup>	0.04±0.01 <sup>a</sup>	0.03±0.01 <sup>a</sup>	0.03±0.01 <sup>a</sup>	-
C13:0	0.02±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	-
C14:0	2.11±0.01 <sup>a</sup>	2.35±0.01 <sup>c</sup>	2.34±0.01 <sup>c</sup>	2.18±0.01 <sup>b</sup>	2.18±0.01 <sup>b</sup>	0.001
C15:0	0.33±0.01 <sup>a</sup>	0.32±0.01 <sup>a</sup>	0.33±0.01 <sup>a</sup>	0.33±0.01 <sup>a</sup>	0.33±0.01 <sup>a</sup>	0.080
C16:0	12.35±0.01 <sup>a</sup>	15.08±0.01 <sup>e</sup>	14.06±0.01 <sup>d</sup>	13.49±0.01 <sup>b</sup>	13.79±0.01 <sup>c</sup>	0.001
C17:0	0.31±0.01 <sup>a</sup>	0.31±0.01 <sup>a</sup>	0.32±0.01 <sup>a</sup>	0.32±0.01 <sup>a</sup>	0.32±0.01 <sup>a</sup>	-
C18:0	4.37±0.01 <sup>e</sup>	4.14±0.01 <sup>c</sup>	3.96±0.01 <sup>a</sup>	4.04±0.01 <sup>b</sup>	4.22±0.01 <sup>d</sup>	0.001
C20:0	0.31±0.01 <sup>a</sup>	0.30±0.01 <sup>a</sup>	0.31±0.01 <sup>a</sup>	0.32±0.01 <sup>a</sup>	0.32±0.01 <sup>a</sup>	-
C22:0	0.18±0.01 <sup>a</sup>	0.17±0.01 <sup>a</sup>	0.18±0.01 <sup>a</sup>	0.20±0.01 <sup>a</sup>	0.18±0.01 <sup>a</sup>	-
C23:0	0.04±0.01 <sup>a</sup>	0.03±0.01 <sup>a</sup>	0.03±0.01 <sup>a</sup>	0.03±0.01 <sup>a</sup>	0.03±0.01 <sup>a</sup>	-
C24:0	0.11±0.01 <sup>a</sup>	0.08±0.01 <sup>a</sup>	0.09±0.01 <sup>a</sup>	0.09±0.01 <sup>a</sup>	0.09±0.01 <sup>a</sup>	-
C14:1	0.02±0.01 <sup>a</sup>	0.03±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	-
C16:1	2.87±0.01 <sup>c</sup>	3.40±0.01 <sup>e</sup>	3.03±0.01 <sup>d</sup>	2.78±0.01 <sup>b</sup>	2.72±0.01 <sup>a</sup>	0.001
C18:1n-9c	26.96±0.03 <sup>d</sup>	25.02±0.01 <sup>c</sup>	24.73±0.01 <sup>a</sup>	24.76±0.01 <sup>a</sup>	24.89±0.01 <sup>b</sup>	0.001
C20:1n-9c	1.30±0.01 <sup>a</sup>	1.16±0.01 <sup>a</sup>	1.05±0.01 <sup>a</sup>	1.02±0.01 <sup>a</sup>	1.04±0.01 <sup>a</sup>	-
C22:1n-9c	0.20±0.01 <sup>a</sup>	0.15±0.01 <sup>a</sup>	0.14±0.01 <sup>a</sup>	0.15±0.01 <sup>a</sup>	0.15±0.01 <sup>a</sup>	-
C24:1	0.36±0.01 <sup>b</sup>	0.25±0.01 <sup>a</sup>	0.24±0.01 <sup>a</sup>	0.25±0.01 <sup>a</sup>	0.24±0.01 <sup>a</sup>	0.001
C18:2n-6c	25.01±0.03 <sup>a</sup>	25.15±0.01 <sup>b</sup>	26.80±0.01 <sup>d</sup>	27.33±0.01 <sup>e</sup>	26.73±0.01 <sup>c</sup>	0.001
C18:3n-6	0.40±0.01 <sup>b</sup>	0.34±0.01 <sup>a</sup>	0.45±0.01 <sup>b</sup>	0.36±0.01 <sup>a</sup>	0.35±0.01 <sup>a</sup>	0.001
C18:3n-3	2.50±0.01 <sup>a</sup>	2.83±0.01 <sup>e</sup>	2.81±0.01 <sup>d</sup>	2.79±0.01 <sup>c</sup>	2.72±0.01 <sup>b</sup>	0.001
C20:2	1.61±0.01 <sup>c</sup>	1.71±0.01 <sup>d</sup>	1.72±0.01 <sup>d</sup>	1.58±0.01 <sup>b</sup>	1.51±0.01 <sup>a</sup>	0.001
C20:3n-3	0.18±0.01 <sup>a</sup>	0.22±0.01 <sup>a</sup>	0.20±0.01 <sup>a</sup>	0.20±0.01 <sup>a</sup>	0.19±0.01 <sup>a</sup>	-
C20:3n-6	0.56±0.01 <sup>a</sup>	0.53±0.01 <sup>a</sup>	0.53±0.01 <sup>a</sup>	0.47±0.01 <sup>a</sup>	0.47±0.01 <sup>a</sup>	-
C20:5n-3(EPA)	1.57±0.01 <sup>a</sup>	1.58±0.01 <sup>a</sup>	1.77±0.01 <sup>c</sup>	1.67±0.01 <sup>b</sup>	1.72±0.01 <sup>c</sup>	0.001
C20:4n-6	0.51±0.01 <sup>c</sup>	0.44±0.01 <sup>a</sup>	0.48±0.01 <sup>b</sup>	0.46±0.01 <sup>b</sup>	0.46±0.01 <sup>b</sup>	0.001
C22:6n-3(DHA)	7.83±0.02 <sup>c</sup>	7.02±0.01 <sup>a</sup>	7.16±0.01 <sup>b</sup>	7.84±0.01 <sup>c</sup>	8.25±0.04 <sup>c</sup>	0.001
C22:5n-3	0.94±0.01 <sup>d</sup>	0.66±0.01 <sup>a</sup>	0.72±0.01 <sup>b</sup>	0.83±0.01 <sup>c</sup>	0.85±0.01 <sup>c</sup>	0.001
C22:2	0.51±0.01 <sup>b</sup>	0.50±0.01 <sup>b</sup>	0.46±0.01 <sup>a</sup>	0.48±0.01 <sup>a</sup>	0.50±0.01 <sup>b</sup>	0.001
ΣSFA	20.16±0.01 <sup>a</sup>	22.84±0.01 <sup>e</sup>	21.67±0.01 <sup>d</sup>	21.03±0.02 <sup>b</sup>	21.51±0.02 <sup>c</sup>	0.001
ΣMUFA	31.70±0.04 <sup>d</sup>	30.00±0.01 <sup>c</sup>	29.21±0.01 <sup>b</sup>	28.98±0.01 <sup>a</sup>	29.06±0.02 <sup>a</sup>	0.001
ΣPUFA	41.60±0.01 <sup>b</sup>	40.98±0.01 <sup>a</sup>	43.09±0.04 <sup>c</sup>	43.99±0.01 <sup>e</sup>	43.70±0.02 <sup>d</sup>	0.001
Omega-3	13.01±0.03 <sup>c</sup>	12.31±0.01 <sup>a</sup>	12.66±0.02 <sup>b</sup>	13.32±0.01 <sup>d</sup>	13.69±0.04 <sup>e</sup>	0.001
Omega-6	26.47±0.03 <sup>a</sup>	26.46±0.01 <sup>a</sup>	28.26±0.01 <sup>c</sup>	28.62±0.01 <sup>d</sup>	28.01±0.02 <sup>b</sup>	0.001
Omega-3/Omega-6	0.49±0.01 <sup>c</sup>	0.47±0.01 <sup>b</sup>	0.45±0.01 <sup>a</sup>	0.47±0.01 <sup>b</sup>	0.49±0.01 <sup>c</sup>	0.001
Omega-9	28.46±0.03 <sup>d</sup>	26.33±0.01 <sup>c</sup>	25.92±0.01 <sup>a</sup>	25.93±0.01 <sup>a</sup>	26.08±0.01 <sup>b</sup>	0.001

SFA: Saturated Fatty Acid, MUFA: Mono Unsaturated Fatty Acid, PUFA: Poly Unsaturated Fatty Acid

Values in the same row with different superscripts are significantly different ( $p < 0.05$ )

Fatty acids with the highest values in the control group and the starvation groups were C18:2, C18:1, and C16:0. In terms of fillet fatty acid composition, no significant differences were found between the starvation groups and control group. The C18:2n-6c, C18:3n-6, C20:4n-6, and C22:5n-3 values in the starvation groups as well as the C16:0, C16:1, C18:1, and C18:3n-3 values in the control group were high ( $p < 0.05$ ).

In terms of the essential fatty acids EPA (C20:5n-3) and DHA (C22:6n-3), the differences between the starvation groups

and control group were significant ( $p < 0.05$ ). As a result of the correlation analysis conducted between the starvation period and EPA, and the starvation period and DHA levels, a significant correlation was detected between the starvation period and DHA levels ( $r = 0.97$ ), whereas the correlation value between the starvation period and EPA was lower ( $r = 0.48$ ) (Fig. 3).

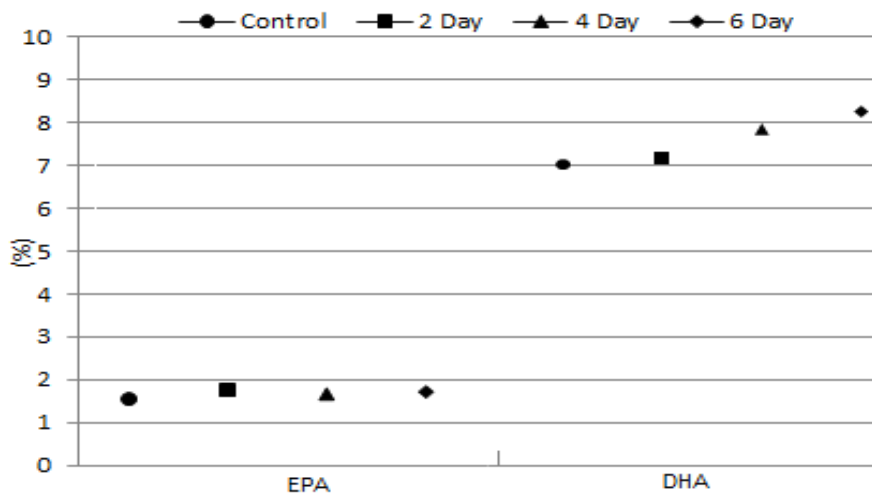


Figure 3: EPA and DHA values and correlation coefficients.

In terms of total saturated fatty acids ( $\Sigma$ SFA) and total monounsaturated fatty acids ( $\Sigma$ MUFA), the highest values were detected in the control group. This was followed by the 2D group. The differences

between the groups were significant ( $p < 0.05$ ). The total polyunsaturated fatty acid ( $\Sigma$ PUFA) values were higher compared to those of the control group ( $p < 0.05$ ) (Fig. 4).



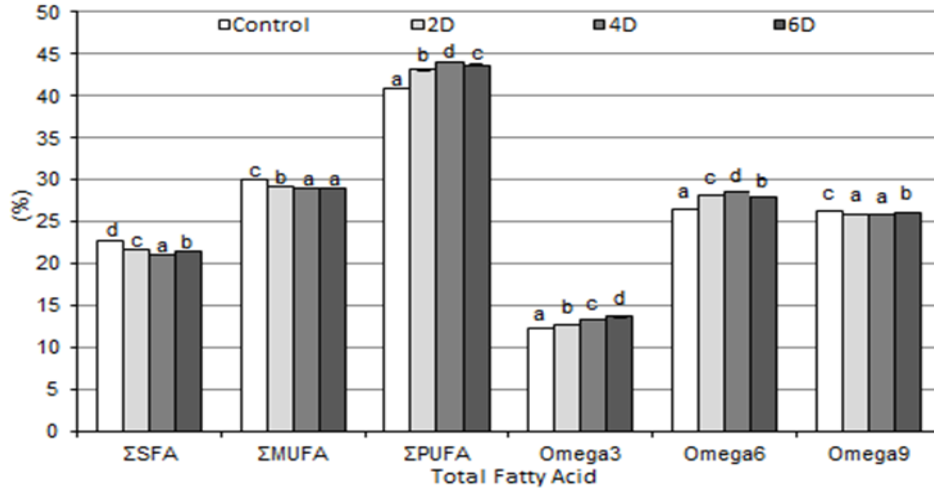


Figure 4: ΣSFA, ΣMUFA, ΣPUFA, Omega-3, Omega-6, and Omega-9 values.

The differences between the groups were significant in terms of Omega-3 fatty acids and the highest value was detected in the 6D group. Omega-6 fatty acid values in the starvation groups were higher than those of the control group, whereas the Omega-9 fatty acid value in the control group was higher than the starvation groups ( $p < 0.05$ ). The differences between the groups were not significant in terms of the Omega-3/Omega-6 ratios ( $p > 0.05$ ).

**Discussion**

It has been known that the starvation period has a negative effect on the growth performance of the fish. In the present study, when examining the effect of the different starvation periods on the growth parameters, it was determined that the starvation groups had lower values in terms of the final weight, specific growth rate, and daily growth parameters compared to those of the control group, and the starvation period had an effect on the growth. In terms of the growth rates in feeding days, the highest value was detected in the 2D group. This result indicates a partial compensatory growth.

In similar studies, it has been reported that the starvation had a significant effect on the growth values (Enien *et al.*, 1998; Akpınar and Metin, 1999; Nikki *et al.*, 2004; Tian and Qin, 2004; Abdel-Tawwab *et al.*, 2006; Türker and Dernekbaşı Yaman, 2006; Baki *et al.*, 2013). Significant decreases were observed in the growth values during the starvation periods (Türker and Dernekbaşı Yaman, 2006; Sevgili, 2007; Kocabaş *et al.*, 2013). The FCR is a parameter that is desired to be low in aquaculture. In this study, the best FCR values based on feed consumption were determined in the control and 2D groups, while the worst FCR values were detected in the 6D group. Furthermore, it was found that long-term starvation had an effect on the feed conversion rate. Chatakondi and Yant (2001) reported that the starvation groups had better FCR values during the refeeding periods compared to those of the continuous feeding group, whereas Wu *et al.* (2004) and Sevgili (2007) reported that the starvation period had no significant effect on the feed conversion rate, and Kim and Lovell (1995), Tian and Qin

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(2004), and Wang *et al.* (2000) all reported that there was no relation between the growth during the starvation periods and feed conversion rates.

It has been detected that, during the feeding period, the average feed consumption and reaction to feed, increased parallel to the starvation period, and the lowest value was detected in the control group. Similarly, it has been reported that in feeding periods, fish having starvation periods consume more feed than the continuously fed groups (Miglavys and Jobling, 1989; Bull and Metcalfe, 1997; Nikki *et al.*, 2004; Eroldoğan *et al.*, 2006a; Eroldoğan *et al.*, 2006b; Sevgili, 2007).

Depending on the starvation period, the feed consumption rates increased while the protein efficacy rates decreased. Although the 2D group had higher feed consumption values compared to those of the control group, it was determined that the 2D group had the best protein efficacy rate values ( $p>0.05$ ), and the differences between the other groups were significant ( $p<0.05$ ). Other studies have reported that there were no significant differences between the starvation groups and other groups in terms of the protein efficacy rates (Sevgili, 2007), while Heide *et al.* (2006) reported that the values obtained in the control group were lower than those of the starvation groups.

In the present study, the 4D and 6D groups had lower values in terms of HSI, carcass yield, and condition factor. Sevgili (2007) has reported that starvation in fish significantly decreased the HSI values while McCue (2010) has reported that the weight loss starts in the digestive system

organs and this is related to the decrease in the HSI values.

Various studies have reported fluctuations in the metabolic activities of the fish and the contents of the stored nutrition during the starvation periods (Jobling, 2010; Baki *et al.*, 2013; Halder and Ali, 2015; Gao *et al.*, 2015). The protein (%), fat (%), dry matter (%), and L/LBM values obtained in the control group were high while, in the starvations groups, the high protein (%), fat (%), dry matter (%), and L/LBM values were obtained in the 2D group. The protein values determined in the groups were close to each other.

The low fat values obtained especially in the starvation groups indicate that the fish obtain their energy needs from the fat sources in their body during starvation periods. The results showed that the starvation period had a significant effect on the biochemical composition of fillet.

Namrata *et al.* (2011) reported that starvation causes an alteration in the biochemical composition of fillet, especially the protein decrease in the muscles were associated with the increase in protein catabolism. In the present study, the lipid contents decreased while the water contents increased in the starvation groups. It has been reported that there was an inverse relationship between the body lipid and water content of the body (Ali and Wootton, 1998; Li *et al.* 2005).

Lower lipid contents in the starvation groups compared to those of the control group were associated with the direct effect of the feeding regimes applied throughout the study on the body lipid compositions of the fish. The fish effectively utilize most of their body lipid

compositions as a source of energy and, therefore, the body lipid values decline. Similar studies have reported that the starvation applications increased the body lipid contents (Akpınar and Aksoylar, 1988; Qian *et al.*, 2000; Zhu *et al.*, 2001; Tian and Quin, 2004).

The body fat contents in the starvation groups were lower than those of the feeding group. The fatty acids analyses showed that there were no significant differences between the fatty acid values. McCue (2010) has reported that fatty acids can vary in spite of the decrease in the body fat rates during the starvation period. Fluctuations in the fatty acid contents the fish use as a source of energy during the starvation periods were observed. The EPA and DHA values increased during the starvation periods. Tidwell *et al.* (1992) and Osako *et al.* (2003) reported that the DHA values increased in fish muscular tissues following starvation periods.

When examining the total fatty acids, it was detected that the  $\sum$ SFA,  $\sum$ MUFA, and omega-9 values were high in the control group while the  $\sum$ PUFA, omega-3, and omega-6 values were high in the starvation groups. Akpınar and Aksoylar (1988) stated that the starvation period had an effect on the fatty acid compositions, while Jezierska *et al.* (1982) stated that the  $\sum$ SFA values in the muscles decreased in trout during the starvation periods. The researchers associated this result with the decrease in the palmitic acid values. Enien *et al.* (1998) have reported that the  $\sum$ SFA and  $\sum$ MUFA values were higher in the starvation groups while the  $\sum$ PUFA, omega-3, and omega-6 values were higher in the fed groups, and starvation had an effect on the fatty acid compositions. Baki

*et al.* (2013) reported that the  $\sum$ SFA values were higher in the starvation groups, whereas  $\sum$ MUFA and  $\sum$ PUFA were higher in the control group. Tidwell *et al.* (1992) have reported that the Omega-3/Omega-6 ratio increased in the starvation groups and this result was associated with the DHA levels.

In conclusion, it was determined that limited feeding applications extend the duration required for reaching the marketing weights, and feeding applications following a two-day starvation period exhibit a compensation feeding effect. Long-term starvation periods had an effect on the feed conversion ratio, condition factor, fillet protein, and fat ratio values, and had no negative effects on the fatty acids composition.

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