

## Effects of feeding frequency on nutrient digestibility and growth performance of rainbow trout (*Oncorhynchus mykiss*) fed a high lipid diet\*

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**Abstract:** The effect of feeding frequency on nutrient digestibility and growth performance was investigated in rainbow trout (*Oncorhynchus mykiss*) fed a high lipid diet. The experimental diet contained 25.2% crude lipid and 40.7% crude protein. Two feeding frequencies were tested: 2 meals per day (Group I) and 6 meals per day (Group II). Fish were fed a restricted daily ration (1.5% of body weight) divided into equal meals.

At the end of the experiment, the weight gain in Group I and II was 84.6% and 90.1%, respectively. There was no statistically significant difference between groups. The crude lipid and protein digestibility values were above 95% in both groups. The feed conversion ratios of Group I and II were 1.28 and 1.21, respectively. There was no significant difference between the 2 groups. Because nutrient digestibility, growth, and feed efficiency were not significantly enhanced when feeding was increased from twice to 6 times daily, feeding twice per day was considered to be sufficient for rainbow trout fed the high lipid diet in this experiment.

**Key words:** Feeding frequency, high lipid diet, digestibility, growth, *Oncorhynchus mykiss*

### Yüksek yağ içerikli yemle farklı yemleme sıklığında yemlemenin gökkuşuğu alabalığında (*Oncorhynchus mykiss*) besin maddelerinin sindirimi ve büyüme performansı üzerine etkisi

**Özet:** Bu çalışmada, yüksek yağ içeriğine sahip bir yemle farklı yemleme sıklığında yemlemenin, gökkuşuğu alabalıklarında besin maddelerinin sindirimi ve büyüme performansı üzerine etkisi araştırılmıştır. Deneme yemi % 25,2 ham yağ ve % 40,7 ham protein içeriğine sahiptir. Denemede 1. gruba 2, 2. gruba 6 kez olmak üzere iki farklı yemleme sıklığı uygulanmıştır. Balıklar, vücut ağırlığının % 1,5'i oranında ve günde iki kez yemlenmiştir.

Denemenin sonunda yüzde ağırlık artışı 1. grupta % 84,6 ve 2. grupta % 90,1 olarak tespit edilmiştir. Gruplar arasında istatistiksel açıdan önemli bir fark bulunmamıştır. Ham yağ ve ham protein sindirilme oranları her iki grupta da % 95 civarında bulunmuştur. Yem değerlendirme sayısı birinci grupta 1,28, 2. grupta 1,21 olarak tespit edilmiştir. Gruplar

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arasında istatistiksel açıdan önemli bir fark bulunmamıştır. Gökkuşuğu alabalığında yağ oranı yüksek yemle beslemede yemleme sıklığı 2'den 6'ya çıkarıldığında besin maddesi sindirilme oranları, büyüme ve yem değerlendirme değerlerinde istatistiksel açıdan herhangi bir artış tespit edilmediğinden, günde 2 kez yemlemenin yeterli olduğu sonucuna varılabilir.

**Anahtar sözcükler:** Yemleme sıklığı, yüksek yağlı yem, sindirilme oranı, büyüme, *Oncorhynchus mykiss*

## Introduction

Feed and labor are the highest variable costs in fish culture and they can be reduced through a suitable feeding management. Good feeding management, including appropriate feeding frequency, can reduce overfeeding and improve efficiency in fish culture (1).

Feeding frequency can affect the feed intake (2) and influence the fish growth (3). The lowest frequency or optimum feeding frequency may be a function of size, age, fish species, water temperature, and food quality (2). When fish are fed using a satiety-based regime, it is difficult to determine the critical point at which fish are satiated. This can cause overfeeding. When fish are overfed, economic losses and deterioration in water quality can occur. Deterioration of water quality suppresses fish growth and causes an increase in fish production cost. A proper feeding frequency and feed amount must be determined to obtain improved fish production and to minimize water pollution (4).

The effects of feeding frequency on fish growth and feed conversion efficiency have been studied for several fish species. These studies show high variable results among and within species because regimes are age and size dependent. No single conclusion can be drawn on the influences of increasing feeding frequency (3).

In order to determine the optimum feeding frequency, from the physiological point of view, all factors that influence feeding frequency must be taken into consideration. They include fish species and size, stomach capacity, digestion time, fish biorhythm, diet quality, and composition (5). The dietary energy level of feed is also important in fish nutrition and affects the fish performance. High-energy diets are based on an optimal balance of nutrients provided by highly digestible ingredients and contain high lipid levels (26%-30%). The protein contents of the high-energy diets can be reduced to 40%. However, ad libitum

feeding with high lipid feeds may also increase the fat level in the fish (6).

The purpose of the present study is to investigate the effects of feeding frequency on nutrient digestibility, growth performance, feed utilization, and body composition of rainbow trout (*Oncorhynchus mykiss*) fed a high lipid diet (25%).

## Materials and methods

The trial was set up in a flow through water system in an indoor facility in the Sinop University Fisheries Faculty in Sinop, Turkey. Rainbow trout were obtained from a commercial farm (Ak Balık Inc.) in Bafra, Samsun, Turkey. During the experimental period, fish were stocked in centrally drained circular fiberglass tanks (water depth: 80 cm; volume: 300 L). Rate of water inflow was adjusted to 4 L/min and supplemental aeration was provided via air stone diffusers. Water quality parameters were monitored daily. The average values for water quality parameters were: temperature  $16.2 \pm 0.2$  °C; DO  $6.5 \pm 0.1$  mg/L; pH 7.5. Photoperiod followed natural conditions during the experimental period. The experiment lasted for 60 days (from 12 April 2004 to 10 June 2004).

Three hundred rainbow trout (initial mean weight:  $95.2 \pm 1.38$  g) were subjected to 24 h fasting. The fish were weighed on a scale to the nearest 0.1 g, and randomly stocked in experimental tanks using 3 replicates per treatment at an initial stocking density of 25 fish per tank. The average initial weight of fish was uniform: there was no statistical difference in weight between treatments ( $P > 0.05$ ). At the start of the trial 10 fish from the initial pool were selected at random. The dorsal fillet of fish were homogenized and analyzed for dorsal muscle composition. At the end of the experiment, 5 fish from each tank were used to analyze the dorsal muscle composition.

Ingredients and composition of the diet are given in Table 1. Diet ingredients were obtained from a local fish feed manufacturer (SIBAL Inc. in Sinop, Turkey). Chromic oxide was incorporated into the test diet at an inclusion level of 0.5% as a marker to assess the apparent digestibility of the prepared diets. Ingredients were thoroughly mixed, homogenized, moistened by the addition of 30% boiling water and pelleted (3.0 mm) by a mincing machine. The pellets were dried at 70 °C for 10 h. Pellets were cut to similar sizes of approximately 5 mm in length. The pellets were stored in plastic bags in a refrigerator at 7 °C during the experiment.

Before starting the fecal collection, fish were acclimated for a week and fed on experimental diet. Two feeding frequencies were applied in this experiment: twice a day in Group I (09:00 and 15:00) and six times a day in Group II (9:00;10:30; 12:00;13:30;15:00, and 16:30) six days per week. Fish

Table 1. Formulation and chemical composition of the experimental diet.

<i>Ingredients (g/kg)</i>	
Peruvian fish meal	340.0
Full fat soybean meal	185.0
Solvent-extracted soybean meal	148.0
Fish oil	185.0
Wheat meal	133.0
Vitamin premix <sup>1</sup>	2.0
Mineral premix <sup>2</sup>	2.0
Cr <sub>2</sub> O <sub>3</sub>	5.0
<i>Proximate composition (%)</i>	
Dry matter	92.6
Crude protein (% dry matter)	40.7
Crude lipid (% dry matter)	25.2
Crude ash (% dry matter)	7.3
NFE+fiber <sup>3</sup>	26.8
Gross energy (kJ/g)	24.3
Ratio of energy (kJ) to protein (g)	59.7
Cr <sub>2</sub> O <sub>3</sub>	0.4

<sup>1,2</sup> Provided per kg of feed: vitamin A, 12500 IU; vitamin D3, 2500 IU; vitamin K3, 10 mg; vitamin B1, 10 mg; vitamin B2, 20 mg; vitamin B6, 15 mg; vitamin B12, 0.03 mg; Vitamin C, 250 mg; niacin, 200 mg; biotin, 1 mg; folic acid, 10 mg; pantothenic acid, 60 mg; Ca, 1000 mg; ethoxyquin, 130 mg; magnesium, 600 mg; potassium, 450 mg; zinc, 90 mg; manganese, 12 mg; Cu, 5 mg.

<sup>3</sup> NFE + fiber: nitrogen-free extract (calculated by difference)

were hand-fed a restricted daily ration (1.5% of body weight) divided into equal meals. Fish were weighed at 2-week intervals and the amount of feed was adjusted accordingly. After each feeding, the tanks were thoroughly cleaned. All possible care was taken during feeding so that no uneaten feed settled down to the tank bottoms. Fecal matter was collected within an hour after the first and last feeding while the tanks were being cleaned: a task accomplished by slow siphoning with a narrow plastic tube (8 mm). Fecal samples from each tank were immediately frozen and stored at -20 °C pending analysis. Chemical analyses were conducted on dried dorsal muscle samples of fish, diet and feces, using standard methods (7). Crude protein was analyzed according to the Kjeldahl method (N × 6.25), crude lipid by difference following petroleum ether extraction in a Soxhlet apparatus, and ash by incineration at 550 °C for 12 h in a muffle furnace.

Chromic oxide in the diet and feces was determined spectrophotometrically at a wavelength of 370 nm after perchloric acid digestion (8). All chemical analyses of feed, feces, and fish samples were carried out in duplicate. Apparent digestibility coefficients for dry matter, nutrients, and energy in the diets were calculated by the following equations:

$$\text{ADC (\%)} = 100 - [100 (\% \text{Cr}_2\text{O}_3 \text{ in diet} / \% \text{Cr}_2\text{O}_3 \text{ in feces}) \times (\% \text{nutrient in feces} / \% \text{nutrient in diet})] \quad (9).$$

$$\text{ADC of dry matter (\%)} = 100 - [100(\% \text{Cr}_2\text{O}_3 \text{ in diet} / \% \text{Cr}_2\text{O}_3 \text{ in feces})] \quad (10).$$

Differences between results were statistically analyzed by one-way analysis of variance (ANOVA) using Minitab Release 13 for Windows.

## Results

Initial and final weight, weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) for the groups are presented in Table 2. Weight gain in the group fed 6 meals a day was slightly higher than weight gain in the group fed twice a day, but differences were not statistically significant ( $P > 0.05$ ). FCRs did not differ significantly between the 2 groups although FCR is slightly lower in the group fed 6 meals a day ( $P > 0.05$ ).

Table 2. Growth performance and feed efficiency in experimental fish.

	Group I 2 meals daily	Group II 6 meals daily
Initial body weight (g)	96.2 ± 1.45	94.3 ± 1.31
Final body weight (g)	177.6 ± 3.41	179.2 ± 2.79
Weight gain (%) <sup>2</sup>	84.6 ± 5.93	90.1 ± 1.65
SGR (%) <sup>3</sup>	1.02 ± 0.13	1.07 ± 0.03
FCR <sup>4</sup>	1.28 ± 0.13	1.21 ± 0.03
PER <sup>5</sup>	1.93 ± 0.11	2.03 ± 0.03

<sup>1</sup>Values are means ± SEM from triplicate tanks.

<sup>2</sup>Weight gain (%) = [(final weight-initial weight) / initial weight] × 100

<sup>3</sup>SGR, Specific growth rate = 100 × [(ln final body weight - ln initial body weight) / 60 days]

<sup>4</sup>FCR, Feed conversion ratio = total diet fed (g) / total weight gain (g)

<sup>5</sup>PER, Protein efficiency ratio = weight gain (g) / protein intake (g)

Apparent digestibility coefficients (ADCs) of dry matter, crude protein, crude lipid, NfE+ crude fiber and gross energy in the groups are presented in Table 3 and the differences were not statistically significant ( $P > 0.05$ ).

Table 3. Apparent digestibility coefficients (%) of the experimental diets.<sup>1</sup>

	Group I 2 meals daily	Group II 6 meals daily
Dry matter	84.8 ± 1.16	83.5 ± 1.14
Crude protein	95.2 ± 0.47	95.0 ± 0.13
Crude lipid	95.8 ± 0.84	95.1 ± 0.52
NfE + Crude fibre	70.6 ± 2.10	67.6 ± 4.57
Gross energy	90.2 ± 2.11	89.7 ± 1.26

<sup>1</sup>The values are given as mean of 3 replicates ± standard error of mean

The dorsal muscle composition of fish fed the experimental diet at different frequencies is shown in Table 4. Fish fed the experimental diet either twice or 6 times a day had identical moisture, crude protein, lipid, and ash contents and there were no significant differences between the 2 groups ( $P > 0.05$ ) (Table 4).

## Discussion

The effects of feeding frequency on fish growth and feed conversion efficiency have been studied for

Table 4. Chemical composition of dorsal muscle of the experimental fish.<sup>1</sup>

	Initial	Group I 2 meals daily	Group II 6 meals daily
Moisture	64.16	71.05 ± 0.87	71.03 ± 0.91
Protein	17.24	17.24 ± 0.57	17.28 ± 0.27
Lipid	7.43	7.55 ± 0.56	7.64 ± 0.15
Ash	1.8	1.51 ± 0.07	1.46 ± 0.06

<sup>1</sup>The values are given as mean of 3 replicates ± standard error of mean.

several fish species, which include catfish (11), rainbow trout (2), milkfish (12), yellowtail flounder (13), flounder (4), Atlantic and shortnose sturgeon (3), gilthead seabream (14), and Florida pompano (15). Results of these studies are widely variable because different feeding frequencies were applied among fish species; within a fish species over time, at different temperatures, water qualities, culture systems, fish size, and feed rations. In this study, a restricted daily ration (1.5% of body weight) was used to investigate the effect of feeding frequency (twice and 6 meals a day) on the nutrient digestibility and growth of fish fed high lipid diets (25%). Increasing the feeding frequency from 2 to 6 meals per day did not significantly affect nutrient digestibility and growth of rainbow trout. In the present study, the results from the apparent digestibility also showed high digestibility of crude lipid and protein (above 95% for both groups). Negative effects of high lipid level on the digestibility of crude lipid and protein were also not observed (Table 3). Bolliet et al. (16) fed rainbow trout (100 g) a high-energy diet (23% lipid-23.5 kJ/g gross energy) and found lower protein and lipid digestibility (88%) than those in our experiment.

In Siberian sturgeon (*Acipenser baeri*), lipid digestibility was reduced from 90% to 68% when dietary lipid was increased from 13% to 23%. In contrast, *Oncorhynchus mykiss* and *Hippoglossus hippoglossus* seem to tolerate high levels of dietary lipid (17). Takeuchi et al. (18) reported that the digestibility of protein and lipid was 98% in rainbow trout, regardless of the lipid contents in the diets (5%-25%). Hung et al. (19) also found that white sturgeon sub-yearlings can utilize diets with high lipid contents (25%-35%) resulting in good growth without major

adverse effects on body composition. These results are in agreement with our investigation, although the fish species are different.

Cho and Jo (20) investigated the combined effect of dietary energy levels (LE-low energy and HE-high energy) and 3 feeding frequencies (1, 2, and 3 meals) on the growth and body composition of Nile tilapia at different water temperatures in winter and summer. They determined that, in winter, weight gain, specific growth rate, feed efficiency ratio, and energy efficiency ratio were significantly affected by dietary energy levels, but not by feeding frequency. This is in agreement with our results with regard to feeding frequency. Further, the chemical composition (moisture, protein, lipid, and ash) of fish muscle was not significantly affected by dietary energy levels and the number of meals. This is also in agreement with our findings. Cho and Jo (20) found that during summer, weight gain and SGR were significantly affected by the number of meals but not by dietary energy levels. Ruohonen et al. (21) reported that at least 3 feedings (satiation feeding regimes) were required for maximum growth in rainbow trout and the proportion of lipid in growth increased with increased number of feedings. However, the protein content was not affected. On the other hand, Einen and Roem (22) reported reduced growth of fish by high dietary lipid levels. In our experiment we did not observe any adverse effect of high lipid levels on growth and feed conversion efficiency.

Wang et al. (23) found that age-0 hybrid sunfish fed 3 and 4 times daily showed greater consumption and growth rates when compared to daily feedings of once or twice; however, the feed conversion ratios (FCR) did not differ among the 4 treatments. The results of Wang et al.'s (23) investigation, with regard to FCR, is in agreement with our findings, as FCR was not affected by the number of meals.

Tsevis et al. (5) determined the optimum feeding frequency for sea bass as 3 meals per day when the time between 2 meals is equal to 6 h. In our experiment, the weight gain, FCR, and apparent nutrient digestibility were also similar for both experimental groups. This indicated that the interval between the meals does not significantly affect the metabolic efficiency of experimental fish in the

present study. In some fish species it was found that the increased growth occurring with an increasing feeding frequency resulted from increased feed consumption (2,24). But in this study we used a restricted daily ration (1.5% of body weight) and did not detect any significant difference in growth among the groups fed 2 meals or 6 meals daily. However, Wang et al. (23) reported increased growth of hybrid bluegills fed multiple meals per day.

Diet composition affects the quality of fish, in particular, high-energy diets may increase lipid content (25), but in this study we did not detect any adverse effect on the body composition of experimental fish. The fat content of fish was 7.43% at the beginning of the experiment and 7.55% and 7.64% at the end of the experiment (for groups I and II, respectively). Hillestad et al. (26) compared diets with 22% and 30% fat that were fed to Atlantic salmon. They reported the same growth response and an insignificant effect on carcass fat content.

According to Wang et al. (23), without knowledge of the relationships between feeding frequency and feeding pattern, over or underfeeding is likely to occur, resulting in food wastage or slowed growth. An optimal feeding regime should be evaluated from the perspective of both the physiology of the species and the economics of production (5). Because nutrient digestibility, growth and feed efficiency were not significantly enhanced by increasing the number of meals from twice to 6 times a day, feeding twice a day seems to be sufficient for rainbow trout (90 g) fed a high-lipid diet (25%) at a fixed ration (1.5% BW).

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