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Full Length Research Paper

The effect of feeding frequency on growth performance and body composition in juvenile rainbow trout (*Oncorhynchus mykiss*) reared in cold seawater

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This study was done to investigate the effects of different feeding frequencies on the growth, feed efficiency and specific growth rate (%) of juvenile of rainbow trout (*Oncorhynchus mykiss,* weight 16.44±0.22 g) rearing in cold sea water conditions (5.8 to 9.3°C; 17 to 18 ppt). Fish were fed on a commercial feed to the apparent satiation twice a day (Control), six times every day (TD6), four times every day (TD4) and three times every day (TD3) for 8 weeks of the trial. The fish fed to satiation in all the experimental groups. Weight gain, growth rate, feed intake and final body weight decreased with reduction of feeding frequency from six times daily to two times daily. The fish fed at TD6 had relatively high apparent net protein retention and better feed conversion efficiency compared with the fish fed at the control, TD4 and TD3. The feeding frequency had significant effect on the crude lipid, crude protein but not moisture and crude ash proximate composition of rainbow trout. It was recommended that six meals per day was the optimal feeding frequency for juvenile rainbow trout reared in cold sea water at 5.8 to 9.3°C, 17 to 18 ppt.

Key words: Feeding frequencies, growth, body composition, sea water, *Oncorhynchus mykiss.*

INTRODUCTION

Feeding at an optimum rate regularly impedes unnecessary feed loss and environmental pollution and is therefore, more efficient. Arranging the time of feeding according to the natural times affects the feed efficiency, feed conversion rate and intake of nourishment elements in a positive way (Bolliet et al., 2001). According to the hypothesis of Wang et al. (1998), over rated feeding frequency enhances the opportunity of feed intake among the fish thus, decreases the individual size variation among the population. For this reason, the feed should be given on an optimal frequency as the fish can get enough feed and represent enough growth rate. However, when the fish is not fed on an optimal frequency, in case of over and long term feeding; the fish develops a larger gut capacity. This situation has been observed in rainbow trout (*Oncorhynchus mykiss*) (Ruohonen and Grove, 1996) and Arctic charr (*Salvelinus alpinus*) (Jobling, 1983). Optimum feeding frequency that fish can show maximum growth rate, is affected by the size of fish and rearing conditions (De Silva and Anderson, 1995). When the fish is fed inadequate or over, growth and feed efficiency can decrease and this situation may increase the cost of fish production besides the quality of water may deteriorate in case of over feeding (Dwyer et al., 2002). Some studies show that moderate restriction in ratio had no effect on feed efficiency (Azevedo et al., 1998; Van Ham et al., 2003). First-feeding fry should be fed a small amount by hand at least ten times per day until all the fish are actively feeding. As the fry grow, frequency of feeding can be gradually decreased to about five times per day. Piper et al. (1982), for rainbow trout (15.1 to 45.0 g) 3 times feeding per day and the rate of 3% of the body weight are recommended.

The aim of this study was to investigate the effect of different feeding frequencies on growth performance, feed utilization and body proximate composition of

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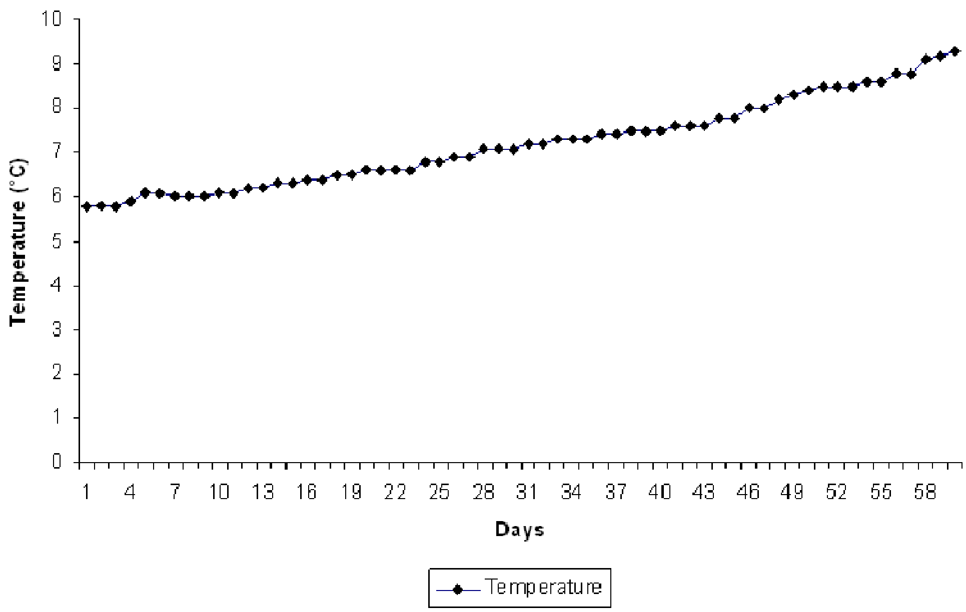

Figure. 1. Water temperature during the experiment.

Table 1. Feeding program for daily feeding in the experiment (● = fed, ○ = not fed).

Feeding group	Time of day								
	0830	1000	1100	1130	1200	1300	1330	1430	1600
Control	●	○	○	○	○	○	○	○	●
TD3	●	○	○	○	●	○	○	○	●
TD4	●	○	●	○	○	○	●	○	●
TD6	●	●	○	●	○	●	○	●	●

juvenile rainbow trout reared in cold sea water temperatures (6 to 9°C; 17 to 18 ppt).

MATERIALS AND METHODS

Experimental fish

Experimental juvenile trout had been obtained from a private commercial trout farm, as 20 fish for each tank total 240 juvenile rainbow trout in average weight of 16.44±0.22g had been stocked into 12 plastic tanks had 60 L volume. The fish had been transferred into the sea water directly without any adaptation. The sea water had approximately 15 L h^{-1} volume of flow been connected into the plastic tank. Water temperature (range of 5.8 to 9.3°C), dissolved oxygen (range 9.3 to 10.5 mg l^{-1}), pH (between 7.8 and 8.4) and salinity (range 0.17 to 0.18%) were monitored regularly with a model WTW multi 340i DO meter. The water temperature had been determined as maximum 9.3°C (in April), minimum 5.8°C (in February) and average 8.3±1.15°C (mean ± SD) (Figure 1).

Experimental diet and rearing conditions

The fish were fed with hand to satiation with commercial extruded pellets (55% protein, 16% fat, 7% ash and 8.5% moisture - Ecobio Industry Co. Turkey, 2 mm diameter) after they were adopted from the sea water. The experiment took totally 60 days, the fish in control group were fed for twice a day (08:30 and 16:00), the fish in TD3 group were fed for three times a day (08:30, 12:00 and 16:00), the fish in TD4 group were fed for four times a day (08:30, 11:00, 13:30 and 16:00) and the fish in TD6 group were fed for six times a day (08:30, 10:00, 11:30, 13:00, 14:30 and 16:00). The fish were fed to satiation for all the experimental groups (twice, three, four and six times a day). The fish were fed according to the feeding schedule in Table 1. It was tried to prevent the feed waste by observing the timing of fish satiety. It was tried to put a stop to time waste which could be originated from feeding by tiring to give feed to the fish being in the same time period at the same time (08:30 or 16:00 feeding time). In this experiment, weighing was done in 4 periods and the total feeding days was counted to be 56 days as there had not been feeding on weighing days.

Calculations and statistical analyses

Feed efficiency, relative growth rate (%), specific growth rate (%), apparent net protein retention (ANPR %) and other growth or feeding parameters were calculated as:

Feed efficiency = 100 × [wet weight gain (g) / dry feed intake (g)]

Table 2. Final body weight, relative growth rate (%), specific growth rate (%) and survival (%) of rainbow trout at different feeding frequencies (mean±S.E, n = 3).

Group	Initial BW (g fish^{-1})	Final BW (g fish^{-1})	Daily growth rate^1 (%)	Relative growth rate^2 (%)	Specific growth rate^3 (%)	Survival (%)^4
Control	16.38 ± 0.14	25.99 ± 0.3^b	0.81 ± 0.11^b	58.63 ± 1.67^b	0.82 ± 0.1^b	88.33
TD6	16.50 ± 0.10	48.10 ± 0.23^d	1.75 ± 0.12^d	191.53 ± 2.38^d	1.91 ± 0.12^d	93.33
TD4	16.33 ± 0.21	34.98 ± 0.46c	1.30 ± 0.13^c	114.21 ± 2.4^c	1.36 ± 0.14^c	90
TD3	16.54 ± 0.24	29.17 ± 0.43^a	0.99 ± 0.13^a	76.30 ± 2.13^a	1.01 ± 0.12^a	90

Within a column, values with different superscripts are significantly different (P < 0.05). ^{1}Expressed as 100 × ((W2-W1) [t ×(W2+W1)/2)]$^{-1}$; ^{2}expressed as 100 × (final BW - initial BW) (initial BW)$^{-1}$; ^{3}expressed as 100 × ((Ln final BW - Ln Initial BW)/Days); ^{4}survival (%) = number fish at harvest / (number fish at harvest + sum of all recovered mortalities) × 100.

Table 3. Feed intake, total protein intake, protein retention, total N intake, ANPR and daily dry energy intake of rainbow trout fed at different frequencies (mean±S.E., n = 3).

Group	Feed/tank (g)	Feed^1 efficiency (%)	Total protein intake^2 (g)	N content in fish^5 (%)	ANPR^6 (%)	Daily dry energy intake (Kcal fish^{-1})
NL	323.39 ± 2.83^b	0.59 ± 0.02^a	177.86 ± 1.56^b	2.75 ± 0.03^a	20.06 ± 0.5^a	5.46 ± 0.05^a
TD6	601.73 ± 7.59^d	1.05 ± 0.03^b	330.95 ± 1.44^d	2.94 ± 0.04^b	37.14 ± 0.19^b	10.15 ± 0.13^d
TD4	368.40 ± 4.83^c	1.01 ± 0.04^b	202.62 ± 2.66^c	2.90 ± 0.06^b	36.10 ± 1.35^a	6.22 ± 0.08^c
TD3	344.49 ± 5.08^a	0.73 ± 0.03^c	189.47 ± 2.80^a	2.85 ± 0.05^{ab}	26.22 ± 0.91^a	5.81 ± 0.08^b

Values are means ± S.D. of three group of trout (n=3), with 15 trout per group. Within a column, values with different superscripts are significantly different (P < 0.05).^{1}Expressed as 100 × [wet weight gain (g) / dry feed intake (g)]; ^{2}expressed as (total feed intake × protein in feed) (100)$^{-1}$; ^{3}expressed as 100 × [(final BW × final protein in fish) – (initial BW × initial protein in fish)] (total feed intake × protein in feed)$^{-1}$; ^{4}expressed as total protein intake 6.25^{-1} total wet weight gain^{-1}; ^{5}expressed as final protein in fish 6.25^{-1}; ^{6}expressed as 100 [((final BW × final protein in fish) – (initial BW x initial protein in fish)) ((total feed intake n^{-1}) × protein in feed)$^{-1}$].

Relative growth rate (%) = 100 × (final BW - initial BW) (initial BW)$^{-1}$

Specific growth rate (%) = 100 × ((Ln final BW - Ln Initial BW)/Days)

Ingredients and feed samples were analyzed by standard methods for moisture (oven drying at 105°C for 24 h), crude protein (N-Kjeldahl × 6.25), Lipid content was determined by 40 to 60°C petroleum ether extraction in a Soxhlet apparatus.

Feed and body composition analysis

Ingredients and feed samples were analyzed by standard methods for moisture (oven drying at 105°C for 24 h), crude protein (N-Kjeldahl × 6.25), lipid content was determined by 40 to 60°C petroleum ether extraction in a Soxhlet apparatus.

Statistical analysis

Differences of all the data were analyzed by one-way analysis of variance (ANOVA). Tukey's multiple procedure was used to compare the differences among mean values. The level for statistical significance was set at 5%. Statistical analyses were run by using MINITAB, version 13.

RESULTS

The final wet weight, relative growth rate (%), specific growth rate (%) and feed efficiency (%) were significantly different among the different feeding frequencies (P < 0.05). Data on final wet body weight, daily growth rate (%), relative growth rate (%) and specific growth rate (%) after the 8 weeks are shown in Table 2. The TD6 groups had the highest final wet weight 48.10 ± 0.23 g. The trout that were fed two times daily had the lowest wet weight 25.99 ± 0.30 g after 8 weeks. Generally, measures of weight gain increased in response to increased feeding frequency. In addition, increasing the number of feeds from 2 to 6 feeds day^{-1} within feeding regime improved survival of trout from approximately, 88.33 to 93.33%. Importantly, increased feeding frequency significantly (P < 0.05) improved apparent net protein retention (ANPR %), total protein intake (g) and daily dry energy intake (kcal fish-^{1}) in the groups (Table 3). Comparisons of simple main effects indicated there were significant differences (P < 0.05) between all feeding frequencies ranked TD6>TD4>TD3>Control in cases.

Body composition and energy content associated with different frequencies are shown in Table 4. In this study in which different feeding frequencies were examined on rainbow trout, data belonged to the average weights obtained as 14 day periods are shown in Figure 2 and specific growth rate is shown Figure 3.

During the experiment, survival rate was changed between 88.33 and 93.33%. The best survival rate was obtained from group TD6 (93.33%).

Table 4. Proximate composition (% wet weight) of rainbow trout fed at different frequencies (mean±S.E. n = 3).

Feeding frequency[1]	Moisture (%)	Crude protein	Crude lipid	Ash	Energy (kJ g^{-1})
Initial	74.4 ± 0.3	16.4 ± 0.4	6.8 ± 0.1	1.8 ± 0.2	6.55 ± 0.05
Control	72.8 ± 0.2^{a}	17.2 ± 0.2^{a}	7.6 ± 0.3^{a}	2.2 ± 0.01^{a}	7.06 ± 0.29
TD6	71.2 ± 0.26^{a}	18.4 ± 0.2^{b}	8.6 ± 0.3^{b}	2.0 ± 0.03^{a}	7.73 ± 0.18
TD4	72 ± 0.82^{a}	18.1 ± 0.31^{b}	8.1 ± 0.22^{ab}	1.9 ± 0.02^{a}	7.47 ± 0.12
TD3	72.4 ± 1.04^{a}	17.8 ± 0.3^{ab}	8.0 ± 0.22^{ab}	1.86 ± 0.04^{a}	7.36 ± 0.26

*Mean values in the same column not sharing a common superscript are significantly different (Tukey's HSD (Honestly Significant Difference) test, $P < 0.05$. [1]Feeding frequencies were: control, two meals per day; TD6, six meals per day; TD4, four meals per day; and TD3, three meals per day.

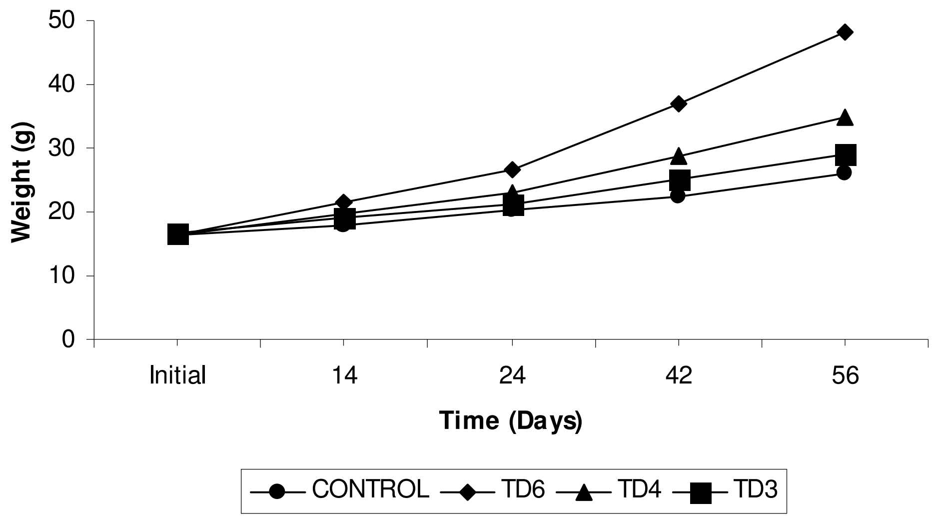

Figure 2. Effect of feeding frequency on the body weight of juvenile rainbow trout (Control; ●), six meals per day (TD6; ♦), four meals per day (TD4; ▲) and three meals per day (TD3; ■) during the experiment.

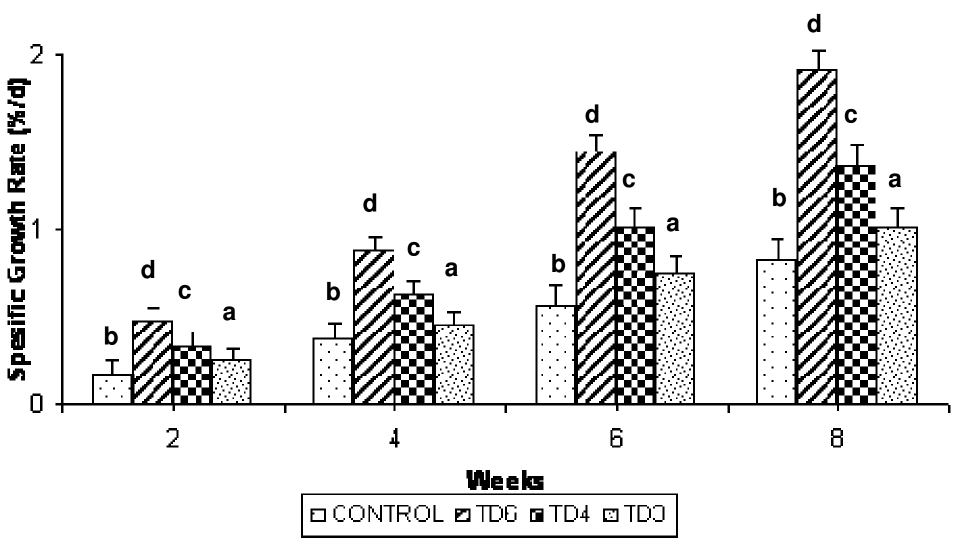

Figure 3. The effect of the different feeding frequency on the specific growth rate (SGR) of rainbow trout during the 8-week study, (control), six meals per day (TD6), four meals per day (TD4) and three meals per day (TD3). Error bars represents SE. Different letters indicate groups that are significantly different ($P < 0.05$) within a sampling period.

DISCUSSION

The feeding frequency applied in our experiment (two, three, four and six rations) significantly improved not only the growth rate, but also the feed efficiency ratio in rainbow trout. The applications of feeding frequencies affected the feed intake and growth positively and decrease the variables in oxygen consuming.

There have been lots of various experiments and comments about the effects of feeding frequency on the growth of fish. It has been explained that the application of feeding for 1 or 3 times daily until satiation to get the maximum growth on salmonids is enough (Ruohonen et al., 1998). The effects of feeding frequency may sometimes result in different even in the members of the same salmonid family and in the Arctic charr (*Salvelinus alpinus*) and rainbow trout (*Oncorhynchus mykiss*) species, which are very close to each other. While an improvement is obtained on Arctic charr when 8 feeding frequency daily is applied, the decreased feeding frequency on rainbow trout improved the growth rate (Linnér and Brännäs, 2001). However, in this trial carried out, the group fed 6 times daily had shown a higher growth rate and feed efficiency values than the other ones. The fish fed with less frequency had adopted to take more feed in one meal. In case of taking this situation longer, there has been an increase on gut capacity (Ruohonen and Grove, 1996). The fish have less feeding frequencies though, the feed transfers faster in the digestive system (Liu and Liao, 1999). For this reason, it is essential to determine optimum feeding frequencies for various fish species on practical applications (Zakes et al., 2006). Continually, juvenile fish need feeding hourly as they need high energy. Over feeding of juvenile fish does not form problems as over feeding of bigger fishes, as the amount of feed given to the juveniles compared with the big fish is proportionally equal to a smaller amount in the water volume of rearing conditions. As the fish grow up, feeding rates, frequencies and protein rates of the feed must be decreased. In this study, the values of the best feed efficiencies were got from groups TD6 and TD4. According to the values of feed efficiencies and feed intake, it has been determined that total protein intake (g), N content in fish (%) and apparent net protein retention (%) values were higher and better in groups TD6 and TD4 than the other groups ($P < 0.05$). On the contrary, Storebakken et al. (1991) did not find any effect on the protein retention when rainbow trout doubled the feed intake from moderate underfeeding to adequate feeding.

The proximate analyses of the fish fed at different feeding frequency are shown in Tables 4. As feeding frequency decreased, the moisture contents of body increased. The feeding frequency had no significant effect on the moist and ash content of body ($P > 0.05$). The crude protein contents of the body in fish fed TD6 was considerably higher than those in fish fed twice daily (control), TD3 and TD4 ($P < 0.05$). As feeding frequency increased, the lipid contents of body and energy in fish increased ($P < 0.05$). The death rates were determined sequentially as control; 88.33%, TD3; 90%, TD4; 90% and TD6; 93.33%. Salmons utilize the energy of feed to get necessary energy for growing and adaptation to the sea. The adaptation to sea water of the fish is a high degree energetic process in which the body energy is used intensively and the plasma metabolism levels and osmotic pressure changes are found together (Sangiao-Alvarellos, 2005; Liebert and Schreck, 2006). The lipid contents of body in fish fed TD6 were significantly higher than two meals per day (control), TD3 and TD4 ($P < 0.05$). The body lipid and protein content of other fish species also increased with an increase in feeding frequency (Yao et al., 1994; Lee et al., 1996).

The amount of feed that the trout need, changes according to the water temperature and the weight of the fish. In optimum rearing conditions, the trout are fed on with high qualified commercial feed formulized according to their needs. As a result of high metabolic rates, juvenile fish consume much more feed as proportion, besides the fish in warm water need more feed than the ones in cold water. Since the fish are poikilothermic (cold-blooded) animals and their metabolic rates change according to the water temperature. For an optimum growth, the optimum water temperature should be between 12.7 and 18.3°C. In this study which is carried out in the sea water ambient (temperature range 6 to 9°C), it was understood that the rainbow trout can show a better feed intake and a better growth with a dispersed feeding series during the day. In various experiments which carried out different species, it has been explained that with the increase of feeding frequency the growth also increases, too (Charles et al., 1984; Sampath, 1984). On the other hand, depending on the increase of the number of feeding frequency, the amount of feed intake in a day increases while the amount of feed intake in a meal decreases (Ruohonen and Grove, 1996). Similar results are observed in this study. The necessary period for salmonids to reach the satiation degree takes 1 h or more. It can be said that the reason for group TD6 consuming more feed than the others is that the stomach are not exactly relaxed or the feeding degree is less than the satiation degree (Ruohonen et al., 1998).

Rainbow trout is the most important cultured fish species in Turkey and widely farmed fish also throughout the world. The production of rainbow trout in Turkey is 75.657 mt in fresh water and 5.229 mt in sea cages of cultured fish (FAO, 2010). In fish farming in the sea, the producer tries to get the best growth rate with the best feeding efficiency by transporting the fish as small as possible into the sea cage. This study was the first to investigate the feeding frequencies at low sea water temperature in juvenile rainbow trout (*O. mykiss,* body weight 16.44 ± 0.22 g). There are still various observations, which can be accepted as contrast, about the effects of water temperature on the digestion rate of fish. While some researchers defined that the water temperature does not have any important effects on digestion (Medale et al., 1991; Iwama et al., 1999), some other researchers (Peres and Oliva-Teles, 1999; Bascinar et al., 2001; Turker, 2006) explain that the digestion rate increases as the temperature increases. It is known that the low temperature causes sluggishness by retarding the digestion speed of the fish (Bailey and Alanara, 2006). It is shown that rearing juvenile rainbow trout at 6 to 9°C in the sea water, giving 6 meals per day clearly enhances the growth. Adoption of this feeding frequency strategy should improve farming efficiency by increasing survival and improving the growth rate in rainbow trout reared in sea water.

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