Effect dietary energy levels and feeding rates on growth and body composition of fingerling rainbow trout (*Oncorhynchus mykiss*)

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Abstract: Growth, feed conversion ratio and fillet composition of rainbow trout (initial weight of 9.99±0.109) were investigated in a 6×2 factorial design experiment employing two levels of digestible energy (DE) (2900 and 3500kcal.kg^{-1}) and six feeding rates (1.0%, 2.0%, 3.0%, 4.0%, 5.0% of the body weight (BW) day^{-1} and to satiation) for 60 days. Specific growth rate (SGR) was highest at 5.0% ration in both levels of digestible energy and decreased in the satiation ration. Regardless of feeding rate, rainbow trout grew more by 35% in DE 3500 kcal kg^{-1}. There was a significant ($P<0.05$) interactive effect of feeding rates and DE on weight gain and feed conversion ratio (FCR). The highest FCR was found in fish fed to satiation (19-21%), while the lowest FCRs, were found in 4%, 3% rations in DE levels of 2900 and 3500kcal kg^{-1}, respectively. There was a significant increase in protein and fat levels and decrease in moisture content of fish fillet ($P<0.05$) as feeding rate and DE increased ($P<0.05$). Condition factor increased when feeding rate and DE increased (14–15%). Feeding rate and DE level proved to be the main differentiating factors in growth, FCR and fillet composition parameters. Values of SGR and FCR plotted against feeding rates allowed the optimum and maximum feeding levels to be determined, which were found to be at 4% and 3%kcal day^{-1} in DE levels of 2900 and 3500kcal kg^{-1}, respectively, for the rainbow trout of l0g average weight.

Keywords: Rainbow trout, Nutrition, Feeding rate, Feed energy

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Introduction

Feeding is one of the most important factors in commercial fish farming because feeding regime may have consequences on both growth efficiency and feed wastage (Tsevis *et al.*, 1992; Azzaydi *et al.*, 2000). Moreover knowledge of the optimum feeding rate is important not only for promoting best growth and feed efficiency, but also for preventing water quality deterioration as a result of excess feeding (Ng *et al.*, 2000; Mihelakakis *et al.*, 2002; Webster *et al.*, 2002). In this context, it is useful to know the optimum feeding rate of the cultured species and how feed efficiency, feed consumption and composition of flesh are affected by it.

Rainbow trout has been introduced to many countries in both northern and southern hemispheres, and has now become extensively distributed throughout many of the freshwater of the world (Stevenson, 1980). It is currently the most important farmed fish in Iran. Economization of fish farming activities in Iran is gaining a momentum in recent years (Salehi, 2008) as aquaculture production is speeding up. Enhancement of feed efficiency is a fundamental factor in economizing fish culture. Feed conversion ratio of rainbow trout under commercial conditions in Iran is often poor and highly variable (ARIFO, 2004). From a practical point of view, rainbow trout farmers have fed their fish according to feeding charts provided by the feed suppliers (Nafisi *et al.*, 2005). These charts prepared under idealized environmental conditions are not always appropriate as stocking rate and size distribution of fish differ from one farm to another. In addition, these charts, which are adopted for water temperature or size of fish, may not be established accurately (Morris & Robert, 2001). Currently, there is few published information about effects of feeding rate on rainbow trout growth. Since feeding rates and digestible energy content of feed are highly inter-related, it is necessary to have a better understanding of the optimum feeding rates of this species according to levels of DE. This study aimed at testing the feeding rates and DE levels on growth performance, feed efficiency and fillet composition of rainbow trout fingerlings.

Materials and methods

This study was conducted in indoor facilities of the Fisheries Department, Faculty of Agriculture and Natural Resources, the Persian Gulf University. Rainbow trout of 9.99±0.109g weight (mean±SD, n=720) obtained from a commercial farm in Kohgiluye and Boyerahmad province. The feeding trials were conducted in 36 circular 150L plastic tanks located in a polyethylene covered saloon. Each tank was stocked with 20 fish and was supplied with continuous aeration by a central aerator (air blower with capacity of $1.3m^3$ min^{-1} and outlet pressure of 110 mbar) and 100% water exchange daily. Daily recordings were taken at 08:00h for temperature, dissolved oxygen, pH and mortality. Dissolved oxygen and pH were maintained at 7.500–7.700 and 7.6-7.7 respectively. Dissolved oxygen and temperature recorded using an oximeter (Oxi 330/set, WTW, Germany) and the pH was measured using a pH meter (pH 330/set–1 WTW, Germany). The average temperature was maintained at 15±2°C, as optimum for growth of rainbow trout (Stevenson, 1980).

Fish were acclimatized to a commercial rainbow trout feed (SFT3) for 7 days prior to starting of the study. The experimental feeds were isonitrogenous with two level of digestible energy. Approximate digestible energy content of the diet was calculated based on the standard physiological values of 4 $kcal.g^{-1}$ protein, 4$kcal.g^{-1}$ CHO and 9 $kcal.g^{-1}$ lipid (Halver, 1976).

A natural photoperiod of 12D:12L was observed and feeding was conducted only during day light (08:00–20:00). Proximate analysis of these feeds is presented in table 1. Six rations of 1.0%, 2.0 %, 3.0%, 4.0%, 5.0% of fish body weight per day (bw day^{-1}) and a satiation feeding level were provided and randomly assigned in triplicate. Fish were fed 3 times a day, making sure that no feed was left uneaten. For satiation feeding, 80g feed was daily provided to the fish, and the leftovers was subtracted from the initial amount.

Table 1: Ingredient and proximate composition of the experimental diets

Ingredients (%)	Diet 1	Diet 2
Norwegian fish meal	46.20	46.20
Wheat flour	23.90	23.90
Wheat gluten	2.50	2.50
Soybean meal	10.00	10.00
Shrimp meal	2.00	2.00
Tuna oil	4.40	4.40
Vitamin premix (1)	3.00	3.00
Mineral premix (2)	3.00	3.00
Filler(3)	5.00	0.00
Proximate analysis (% dry matter basis)		
Crude protein	43.56±0.35	43.40±0.20
Crude lipid	8.80±0.26	13.73±0.25
Crude fiber	2.00±0.00	2.50±0.50
Ash	32.50±0.50	20.83±0.28
N – free extract(4)	6.36±0.55	11.23±1.29
Digestible energy ($kcal.kg^{-1}$)	2869.33±20.52	3521±10.11

1. Vitamin premix contained the following amount (g/kg premix): L- ascorbic acid, 121.2; Dl-α-tocopheryl acetate,18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7;myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.
2. Minerial premix contained the following ingredients (g/kg premix): $MgSo_4$ $.7H_2O$, 80.0; $NaH_2PO_4.2H_2O$, 370.0; KCl, 130.0; ferric citrate, 40.0; $ZnSO_4.7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3.6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$. 0.01; $MnSO_4.H_2O$, 2.0; $CoCl_2.6H_2O$, 1.0.
3. Fine sand used as a non-effective ingredient in diets.
4. Calculated by difference (100- crude protein – crude lipid – crude fiber – ash).

All fish in each tank were individually weighed at every 15 days interval, and daily feeding rations were also adjusted every 15 days. Fish were not fed 12 h prior to samplings. The tanks were scrabbed and washed during each weighing. At the end of the experiment, all fish in each tank were individually weighed to determine survival (%), total length for condition factors, also five fish from each tank were randomly captured as pool sample and sacrificed for fillet composition analysis. Fillets were ground and homogenized in a blender and were stored at –20°C and thawed at 4°C for 24h prior to analysis. Moisture, crude fiber, ash and peroxide value were determined by the methods given in the (AOAC, 1990). Total nitrogen (crude protein = % N×6.250) was determined by the semi–micro Kjeldhal technique (Munro & Fleck, 1969). Crude lipid was determined by the trichlorofluoromethane method (Korn & Macedo, 1973). Acid insoluble ash was determined on ashed samples according to the method of Pearson (1970).

Differences between treatments were tested for significance ($P<0.05$) by Duncan's Multiple Range test and using ANOVA with a sequential sum of squares (type 1 SS) model.

Results

Survival in both digestible energy levels (DE) were 100% and no mortality was seen throughout the experiment. As expected, with increase in feeding rate and DE level, final body weight and specific growth rate (SGR) of rainbow trout increased (Table 2 and Fig. 1). Overall, the fish that were fed higher DE level (3500kcal.kg^{-1}) showed higher body weight (BW) and specific growth rate (SGR), resulting in higher weight gain compared to the fish fed lower DE level (2900kcal.kg^{-1}) (Tables 2 and 3a and 3b). The maximum weight gain was attained at the feeding rate of 5.0% bw day^{-1} in both DE levels. Fish fed lower DE level (2900kcal.kg^{-1}) had about 6-7% lesser weight gain at each feeding rates compared to the higher DE level.

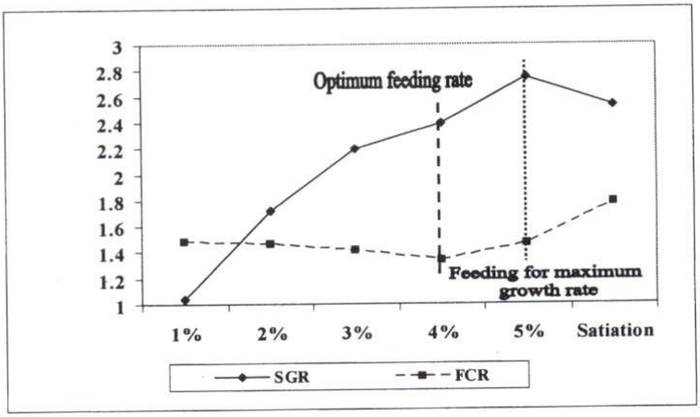

Figure 1: Interaction of DE 2900kcal.kg^{-1} and daily feeding rate on FCR and SGR

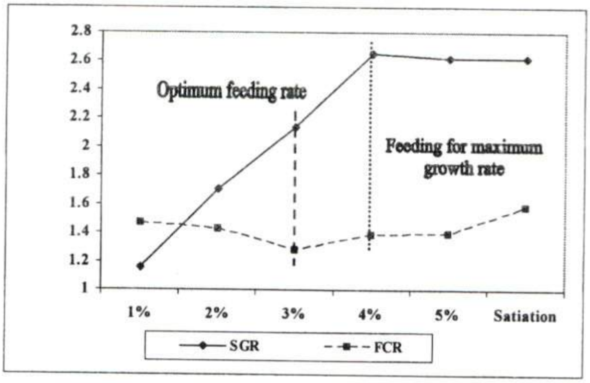

Figure 2: Interaction of DE 3500kcal.kg^{-1} and daily feeding rate on FCR and SGR

Table 2: Growth performance of rainbow trout at different feeding rates and DE levels (X± SD)*

Group	Feeding rate (%bw day^{-1})	BW**	SGR***	FCR****	PER*****
DE	1	18.658 ± 1.019^{h}	1.042 ± 0.084^{h}	1.479 ± 0.003^{e}	1.552 ± 0.004^{h}
(2900 kcal.kg^{-1})	2	27.763 ± 0.664^{f}	1.722 ± 0.066^{f}	1.467 ± 0.002^{d}	1.565 ± 0.002^{g}
	3	36.267 ± 1.179^{e}	2.189 ± 0.039^{e}	1.411 ± 0.005^{f}	1.627 ± 0.006^{e}
	4	42.860 ± 0.909^{d}	2.387 ± 0.036^{d}	1.333 ± 0.004^{i}	1.722 ± 0.006^{b}
	5	50.941 ± 0.622^{a}	2.745 ± 0.005^{a}	1.464 ± 0.004^{d}	1.568 ± 0.004^{g}
	Satiation	45.333 ± 0.577^{c}	2.525 ± 0.019^{e}	1.783 ± 0.003^{a}	1.287 ± 0.002^{j}
	1	20.028 ± 0.541^{g}	1.158 ± 0.027^{g}	1.470 ± 0.003^{d}	1.567 ± 0.003^{g}
	2	27.663 ± 1.417^{f}	1.704 ± 0.037^{f}	1.425 ± 0.004^{e}	1.616 ± 0.005^{f}
DE	3	36.583 ± 0.509^{e}	2.137 ± 0.035^{e}	1.278 ± 0.003^{j}	1.803 ± 0.004^{a}
(3500 kcal.kg^{-1})	4	42.215 ± 0.605^{b}	2.649 ± 0.023^{b}	1.382 ± 0.003^{h}	1.667 ± 0.003^{c}
	5	48.297 ± 0.257^{b}	2.169 ± 0.018^{b}	1.391 ± 0.004^{g}	1.656 ± 0.004^{d}
	Satiation	48.148 ± 1.349^{b}	2.622 ± 0.070^{b}	1.578 ± 0.003^{b}	1.460 ± 0.003^{i}

Average initial body weight was 9.998±0.109g.

** Whole – body wet weight

*** Specific growth rate = 100 (L_n final body weight – L_n initial body weight/days)

**** Feed conversion rate = Average of food consumed in dry weight/average gain in live weight (Ahmad Ali, 1993)

***** Protein efficiency ratio =100 (Final total body protein–initial total body protein)/ total dietary protein fed (Kang *et al.*, 2004).

Feed conversion ratio (FCR) was significantly influenced by feeding rate and DE level. The lower DE level was associated with higher FCR than that of the higher DE level, regardless of the feeding rate (Table 3c). The lowest FCR was found at 4.0% bw day^{-1} in lower DE level and 3.0% bw day^{-1} in higher DE level. FCR significantly increased at rations below 3.0% bw day^{-1} in lower DE level and above 4.0% bw day^{-1} in higher DE level (Table 2). Consequently, DE level ($P< 0.0003$) and ration ($P<0.0001$) had significant effects on FCR. Protein efficiency increased with increase feeding rate and DE level. The highest PER was found at 3.0% bw day^{-1} in higher DE level and lowest PER was found at 4.0% bw day^{-1} in lower DE level.

There were significant interaction of DE levels and feeding rate ($P<0.001$) on SGR (Table 3b, $P<0.0011$), and significant interaction was found between them (Table 3b, $P<0.0010$). Optimum and maximum feeding rates for SGR were found at 4.0% and 5.0% bw day^{-1} rations in lower DE level and 3.0% and 5.0% bw day^{-1} rations in higher DE level (Table 2 and Figs. 1 & 2), respectively.

The fish fed higher DE level (3500 kcal kg^{-1}) contained more lipid and protein in their fillet compared to those fed lower DE level ($P<0.05$). Protein content of the fillet in both DE levels was higher at 4.0% bw day^{-1} ration and the lipid content was higher at 5.0% bw day^{-1} ration ($P<0.05$). Overall, there was an interactive effect of feed DE levels and feeding rate on fillet protein ($P<0.01$) and fillet lipid ($P>0.33$). Fillet moisture content decreased with increase in feeding rate and DE level ($P<0.05$), but no interactive effect of DE level and feeding rate was found ($P>0.12$).

Table 3: Results of growth performance of rainbow trout fed two levels of DE and different levels of rations at 15°C

Factors	*df*	SS*	F value	Prob > F
(a) BW				
Feeding rate	5	4389.833	1402.360	0.001
Feed energy level	1	16.448	26.270	0.001
Feeding rate *	-------	-------	-------	-------
Feed energy level	5	69.482	22.200	0.001
Error	22	13.773	-------	-------
(b) SGR				
Feeding rate	5	11.32511	1389.630	0.0001
Feed energy level	1	0.02336	14.330	0.0011
Feeding rate *	-------	-------	-------	-------
Feed energy level	5	0.14286	17.410	0.001
Error	22	0.03422	-------	-------
(c) FCR				
Feeding rate	5	0.44271	7179.000	0.0001
Feed energy level	1	0.04264	3457.480	0.0001
Feeding rate *	-------	-------	-------	-------
Feed energy level	5	0.06129	993.910	0.0001
Error	22	0.00027	-------	-------
(d) PER				
Feeding rate	5	0.44783	5190.700	0.0001
Feed energy level	1	0.05055	2929.580	0.0001
Feeding rate *	-------	-------	-------	-------
Feed energy level	5	0.06116	708.860	0.0001
Error	22	0.00037	-------	-------
(e) CF				
Feeding rate	5	0.12623	29.260	0.0001
Feed energy level	1	0.00719	8.340	0.0085
Feeding rate *	-------	-------	-------	-------
Feed energy level	5	0.00399	0.920	0.4836
Error	22	0.01898	-------	-------

*** Sum of squares**

Discussion

Increase in the growth of rainbow trout as the results of increase in feeding rate in the current study corresponds with the results reported by other researchers which studied several species (De Silva *et al.*, 1986; Hung & Lutes, 1987; Xiao–Jun & Ruyung, 1992; Adebayo *et al.*, 2000; Mihelakakis *et al.*, 2002; Eroldogan *et al.*, 2004). Growth is not the only parameter that has to be taken into consideration when trying to determine optimal feeding rate for a given fish species. It is known that feed efficiency percent decreases curvilinearly with increasing feeding rate (Hung *et al.*,1993; Mihelakakis *et al.*, 2002; Eroldogan *et al.*, 2004). Under the condition of low feeding rate, fish tend to optimize their digestion to extract more nutrients more efficiently, thus decreasing the FCR (Zoccarato *et al.*, 1994). In the present study, the mean FCR of rainbow trout increased with increasing feeding rate. The lowest FCR (1.28) was found at 3.0% bw day^{-1} feeding rate at higher energy level (3500kcal.kg^{-1}), while fish fed 5.0% bw day^{-1} feeding rate displayed the best growth and higher FCR in both DE levels (1.78,1.58), respectively for lower DE and higher DE. Similarly Puvanendran *et al.* (2003) indicated that in yellow tail flounder minimum FCR was obtained at ration levels (1.0% bw day^{-1}) below those at which maximum growth occurred (3.0% bw day^{-1}). This can be explained by the lower energy and protein retention at satiation feeding when high amount of feed is consumed (van Ham *et al.*, 2003). The FCR results of this study on rainbow trout are generally in agreement with earlier specified works.

It has been shown that feeding fish at different levels of energy can result in differences in both feed intake and efficiency. These parameters are complementary to each other, that is, high feed intake results in a high FCR (De Silva & Perera, 1976; Partridge & Jenkins, 2002). At the end of 60 days, considering the result of FCR, fish on lower DE consumed more feed (higher FCR) than those on higher DE. The amount of feed consumed by fishes at satiation treatment in lower DE was higher than higher DE, with the resulting FCRs of 1.78 and 1.58, respectively. Generally, a decrease in feed conversion ratio with increasing level of DE was demonstrated. Similar results were also shown in PER (Tables 2 and 3).

During the past few years, production of rainbow trout had been progressively increased in Iran. Therefore, it is important to understand the effects of feeding rate on fish fillet composition and flesh quality. The proximate composition of fish fillet (Webster *et al.*, 2002), whole body and carcass (Hung *et al.*, 1993; Ng *et al.*, 2000; Mihelakakis *et al.*, 2002) are affected by feeding rate. Generally, under suboptimal feeding conditions, fish retain more protein in the muscle (Austreng *et al.*, 1987; Hung *et al.*, 1993; Ng *et al.*, 2000); however, body lipid is reduced when the feeding rate is lowered (Mihelakakis *et al.*, 2002; Van Ham *et al.*, 2003). Ng *et al.* (2000) in tropical bagrid catfish (fed up to 3.0% bw day^{-1}) and Hung *et al.* (1993) in stripped bass (fed above 0.5% bw day^{-1}) also observed that carcass and body lipid content of the fish increased with the increasing in feeding rate. In this study we observed an increase in fillet lipid with an increase in feeding rate and DE. An increase in body lipid deposition as the results of increase in feed energy have also been reported (Reinitz *et al.*, 1978; Austreng, 1979; Buckley & Groves, 1979; Cowey, 1981,1994; Watanabe *et al.*, 1979). These researchers reported that an excessive energy intake at moderate dietary protein levels leads to the accumulation of fat in fish with subsequent changes in carcass composition. Fillet protein increased with increase in DE and feeding rate and reached to the best situation at 3.0%, 4.0% bw day^{-1} ration in higher DE and lower DE, respectively. This result show the best protein efficiency when the FCR is in the best situation, although (Gouveia, 1992) reported that with the increase in fish body lipid there was a small but significant decrease in body protein deposition. In this study with increase in fillet protein and lipid, body moisture deposition was decreased. This relationship between body lipid, protein and moisture has also been reported earlier (Papoutsoglou & Paparaskeva-Papoutsoglou, 1978; Reintz *et al.*, 1978; Buckley & Groves, 1979; Weatherley & Gill, 1983).

This study has demonstrated that there was an interaction between level of feed energy and feeding rate in rainbow trout. Both parameters are important to determine the growth performance and feed conversion ratio of rainbow trout. Although higher feeding rates (5.0% bw day^{-1} and at satiation) provide better growth for rainbow trout fed two levels of DE (2900 and $3500kcal.kg^{-1}$), FCR was increased. The better growth rates accompanied with higher FCR values are generally indicators of overfeeding where feed wastage may cause water quality problems and increased costs as well. In conclusion, the current study demonstrated that the optimum feeding rate for rainbow trout of average weight 10g, at 15°C is 4.0% bw day^{-1} in $2900kcal.kg^{-1}$ DE and 3.0% bw day^{-1} in $3500kcal.kg^{-1}$ DE.

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