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Full Length Research Paper

Compensatory growth assessment by plasma IGF-I hormone measurement and growth performance in rainbow trout (*Oncorhynchus mykiss*)

Ruhollah Rahimi[1]*, Mehrdad Farhangi[2], Bagher Mojazi Amiri[2], Fatemeh rezaie[2], Parisa Norouzitallab[2] and Afshin Afzali[2]

[1]Department of Fisheries, Faculty of Marine Sciences, Chabahar Maritime University, Chabahar, 99717-56499, Iran.
[2]Department of Fisheries and Environment, Faculty of Natural Resources Engineering, Tehran University, Karaj, 31585-4314, Iran.

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This study aimed to show the difference in compensatory growth (CG) with different starvation and feeding periods replications, depending on the IGF-I hormone level in the blood. There were 4 treatments in 3 replications. Other indexes like food coefficient ratio (FCR), specific growth rate (SGR) and daily food intake were also examined during the experiment. Fish were fed twice a day *ad libitum* as follows during the 65 days. Treatment A (TA): control treatment, continues feeding. Treatment B (TB): 4 weeks of starvation and 5 weeks of re-feeding. Treatment C (TC): 3 weeks of starvation and 5 weeks of re-feeding. Treatment D (TD): 2 weeks of starvation and 5 weeks of re-feeding. Each tank contained 23 fishes in each unit with an initial mean weight (SD) of 47.19 ± 0.42 (g). Blood was sampled in IGF-I hormone concentration at the beginning of the experiment, at the end of the starvation period and every 12 days in re-feeding periods. There was no significant difference between the treatments in FCR ($P > 0.05$). TB and TC had significant difference ($P < 0.01$) in comparison with other treatments in SGR, but no significant difference was observed among them ($P > 0.05$). IGF-I concentrations came down in comparison with control treatment at the end of the starvation period (Day 29) ($P < 0.001$), but no significant difference was observed among the treatments at the end of the re-feeding period ($P > 0.05$). According to the results, TB and TC showed more indexes of CG in comparison with TA and TD. Still IGF-I cannot show the quality of CG alone and other growth relating physiological elements in different feeding diets and regimes will be evaluated in future studies.

Key words: Compensatory growth, food coefficient ratio, food intake, IGF-I, rainbow trout, special growth ratio.

INTRODUCTION

Most teleost fish species require high levels of dietary amino acids (300 – 600 g/kg; Cowey, 1995) which comercially meet with fish meal-based feed. The sustainability of this practice, which requires large inputs of wild fish for feed, has been questioned (Naylor et al., 2000). Rainbow trout, *Oncorhynchus mykiss*, is one of the most demanded fishes all around the world both from the production and consumption point of view. The most cost effective part of trout farming belongs to food due to the high protein requirement. Feeding practices can have significant effects on trout farming expenses and productivity. True satiation feeding can be difficult to achieve economically in ponds, because many factors can affect daily food intake. Variations in feeding response can result either in over-feeding or under-feeding of fish, which in turn can have a negative effect on the production cost (Reigh et al., 2006). Since compensatory growth (CG) is characterized by accelerated growth and improved feed conversion, the response has the potential to improve cultivation of this economically valuable fin fish. CG is referred to as a period of growth that exceeds normal rates after animals are alleviated of certain growth-stunting conditions (Picha et al., 2006).

*Corresponding author. E-mail: r_rahimi6083@yahoo.com. Tel: 0098545-2224264. Fax: 0098545-2221025.

Compensatory growth has been demonstrated in many, but not all, teleosts and appears to be dependent on a variety of factors including the degree of growth suppression and catabolism prior to the response (for review see Ali et al., 2003). There are several reports of CG in fishes, such as brown trout, *Salmo trutta* (Alvarez and Nicieza, 2005), Atlantic halibut, *Hippoglossus hippoglossus* (Heide et al., 2006), gilthead sea bream, *Sparus auratus* (Montcerrat et al., 2007), channel catfish, *Ictalurus punctatus* (Reigh et al., 2006), *Vimba vimba* (Mycskowsky et al., 2006), Prussian carp, *Carassius auratus gibelio* (Misaila et al., 2007) and rainbow trout, *O. mykiss* (Nikki et al., 2004). Generally, CG may occur due to the endocrine system alterations (Hornick et al., 2000); however, little is known about the endocrine control of CG in teleosts (Picha et al., 2006). Various studies had reported hormones as growth controlling factors (Jones and Clemmons, 1995; Mommsen, 1998), one of the main being insulin-like growth factor-I (IGF-I) (Duan, 1998; Perez-Sanchez and LeBail, 1999). The growth hormone (GH) insulin-like growth factor (IGF) axis is central to the control of growth in teleost fishes, as well as in other vertebrates (Jones and Clemmons, 1995; Oksbjerg et al., 2004; Wood et al., 2005). Insulin-like growth factor-I (IGF-I), a 70 aa polypeptide produced primarily in the liver, is involved in cell differentiation and proliferation and ultimately body growth (Moriyama et al., 1994). Although endocrine IGF-I of hepatic origin is thought to account for the majority of somatic growth, autocrine and paracrine effect may also play a significant role (Chauvigne et al., 2003). The foremost role of IGF-I is to regulate development and growth by mediating growth hormone (GH) action. However, it has other biological effects, such as direct effects on cell growth, differentiation and metabolism (Banos et al., 1999). IGF-I has been reported to be affected by the nutritional factors.

There are some researches on using hormones as growth markers (Perez-Zanchez et al., 1999). Dyer et al. (2004) studied the effects of different diets and feeding regimes in different fin fishes such as barramundi (*Lates calcarifer*), Atlantic salmon (*Salmo salar*) and Southern Bluefin tuna (*Thunnus maccoyii*) by measuring the plasma IGF-I concentration and suggested that IGF- I can be a good index for the assessment of diets and feeding regimes. There are not many studies on CG ranges which evaluate the physiological indexes, especially by IGF-I hormone which can be a fast and easy index to know the CG performance. Therefore, the present study was conducted to assess compensatory growth by plasma IGF-I concentration that are affected by different starvation and re-feeding periods.

MATERIALS AND METHODS

Experimental animals and design

Fingerlings rainbow trout procured from Dr Motamed Farm (Iran, Karaj) were transported with proper aeration to the Nutrition Laboratory of Fisheries and Environmental Department, Tehran University, Karaj, Iran. The fishes were acclimatized to the laboratory conditions for about 2 weeks during which they were fed with control diet. The feeding trial was conducted in uniform tanks (with semi re-circulation system) of 100-L capacity (with water volume of 90 L). Groups of 23 fishes (average weight: 47.19 g /fish) were stocked in 12 tanks, which were randomly distributed in four treatments each with three replicates. Treatment A (TA): control treatment, continues feeding; treatment B (TB): 4 weeks of starvation and 5 weeks of re-feeding; treatment C (TC): 3 weeks of starvation and 5 weeks of re-feeding; treatment D (TD): 2 weeks of starvation and 5 weeks of re-feeding. Fish were fed with dry pellets (Chineh GFT-1 3.5 mm; 37% protein, 14% fat, 20% carbohydrate, 12% ash, 9% humidity according to the manufacturer). Feeding was done twice daily *ad libitum*. The experiment was conducted in 12:12 h light–dark cycles. Water exchange (30%) was carried out daily. Water quality parameters (temperature, 15.2; pH, 7.6; dissolved oxygen, 8.2) were checked daily using HACH^{TM} digital portable and were found within the optimum range.

Growth study

Fishes in each tank were bulk weighed twice, first after the starvation period and second after the feeding period. Growth performance of fishes was evaluated in terms of weight gain, food conversion ratio (FCR) and daily feed intake (FI) based on the following standard formulae:

Weigth gain % = (Wf – Wi) /Wi × 100

SGR (% per day) = (lnWf – lnWi) / t x 100; where Wf is the final wet weight, Wi is the initial wet weight and t is the number of days.

FCR = total dry feed intake (g) /wet weight gain (g)

FI (%) = Feed intake (g) / biomass (g) day $^{-1}$ × 100.

Blood sampling

Blood was sampled for IGF-I hormone concentration examination at the beginning of the experiment, at the end of the starvation period and every 12 days in re-feeding periods. To minimize the effect of handling stress, fish were anesthetized with clove pink extract at a dose of 2 ml/l and blood samples were collected taken from the caudal vein using syringe, which was previously rinsed with ethylene-diamine-tetra-acetic acid, EDTA (as an anticoagulant). Blood collected was then transferred immediately to an eppendorf tube containing a thin layer

Table 1. Growth, food intake and food coefficient ratio, Initial weight, weights at the end of fasting periods, specific growth rates, Weight gain, survival rate, FCR, daily food intake results.

Index	TA	TB	TC	TD	p
Initial weight (g ± sd)	46.86 ± 0.056 [a]	47.58 ± 0.11[a]	46.79 ± 0.76 [a]	47.52 ± 0.33 [a]	0.09
Weight after fasting (g ± sd)	89.36 ± 1.16 [a]	43.22 ± 0.22 [d]	53.48 ± 1.07 [c]	62.55 ± 0.31 [b]	0.000
Weight gain (g ± sd)	59.24 ± 5.50[a]	57.89 ± 4.15[a]	62.42 ± 6.23[a]	62.04 ± 2.45[a]	0.62
Final weight (g ± sd)	148.61 ± 6.61 [a]	101.11± 4.22 [c]	115.91 ± 7.28 [b]	124.59 ± 2.7 [b]	0.000
SGR	1.53 ± 0.08 [c]	2.47 ± 0.05 [a]	2.34 ± 0.14 [a]	2.08 ± 0.05 [b]	0.000
Daily feed Intake (%)	1.66 ± 0.08 [b]	2.15 ± 0.27 [a]	2.16 ± 0.09 [a]	2.01 ± 0.15 [ab]	0.02
FCR	1.29 ± 0.08 [a]	1.08 ±0.01 [a]	1.24 ± 0.27 [a]	1.15 ± 0.02 [a]	0.32
Survival (%)	97.10 ± 0.02 [a]	98.55 ± 0.02 [a]	97.10 ± 0.02 [a]	98.55 ± 0.02 [a]	0.80

Treatment A (TA): Control treatment, continuous feeding. Treatment B (TB): 4 weeks of starvation and 5 weeks of refeeding. Treatment C (TC): 3 weeks of starvation and 5 weeks of refeeding. Treatment D (TD): 2 weeks of starvation and 5 weeks of refeeding.
Each tank was containing 23 fishes in each unit with an initial mean weight (SD) 47.19 ± 0.42.

of EDTA powder and shaken well to prevent hemolysis of blood. The tubes were then centrifuged at 3000 g for 10 min and the plasma was collected and stored at -80°C until radio-immunoassays (RIA) were performed.

IGF-I assay

Plasma IGF-I level was measured by RIA, using human recombinant IGF-I as standard and rabbit anti-human IGF-I antibodies as antiserum (Pérez-Sánchez et al., 1994) and which had been validated for brown trout plasma (Baños et al., 1999).

Statistical methods

A Kolmogorov–Smirnov test was used to assess the normality of distributions. Data of IGF-I, growth and nutritional parameters were compared using one-way analysis of variance (ANOVA) and Tukeys multiple range test. Statistical significance was accepted at $P < 0.05$ levels. Minitab 13.0 software was used for statistical analysis.

RESULTS

Growth performance and survival of rainbow trout

Results (Table 1) showed that final weights, SGR and daily food intake were significantly ($P < 0.05$) decreased by fasting periods, while there were no differences in comparison altogether. Growth data (Table 1) after re-feeding periods showed that weight gain of rainbow trout fingerlings were not significantly different ($P > 0.05$) in all of the groups. Results of SGR (specific growth rates) were significantly ($P < 0.01$) improved by re-feeding practice. The highest SGR was recorded in treatments B and C groups, and lowest SGR were recorded in control (without fasting period). Food coefficient ratios data (Table 1) did not show significant difference between groups ($P > 0.05$), but daily food intake percentage data (Table 1) showed differences between treatments B and C in comparison with control group ($P < 0.05$). The highest daily food intake percentage was recorded in treatments B and C groups and lowest daily food intake percentages were recorded in control (without fasting period).

IGF-I

Results (Figure 1) showed IGF-I concentrations and their changes during fasting and re-feeding periods. The plasma IGF-I levels of the treatments TB, TC and TD were significantly ($P < 0.05$) decreased during fasting; however, no significant differences were observed between the fasting groups. In contrast, the plasma IGF-I level in groups with re-feeding started to increase gradually, reaching the control level at the end of the re-feeding periods.

IGF-I and food intake correlation

Plasma IGF-I and food intake data (Figure 2) showed significant correlation ($r^2 = 0.81$, $P = 0.000$) between them.

DISCUSSION

Weight gain was not significantly different between groups. The SGR results from this study showed that rainbow trout fingerlings are affected by fasting periods. SGR was significantly better for the test groups than for control fish in refeeding period. SGR for the TB and TC groups were higher than for the TD group. Fasting periods influenced on growth rate in refeeding periods

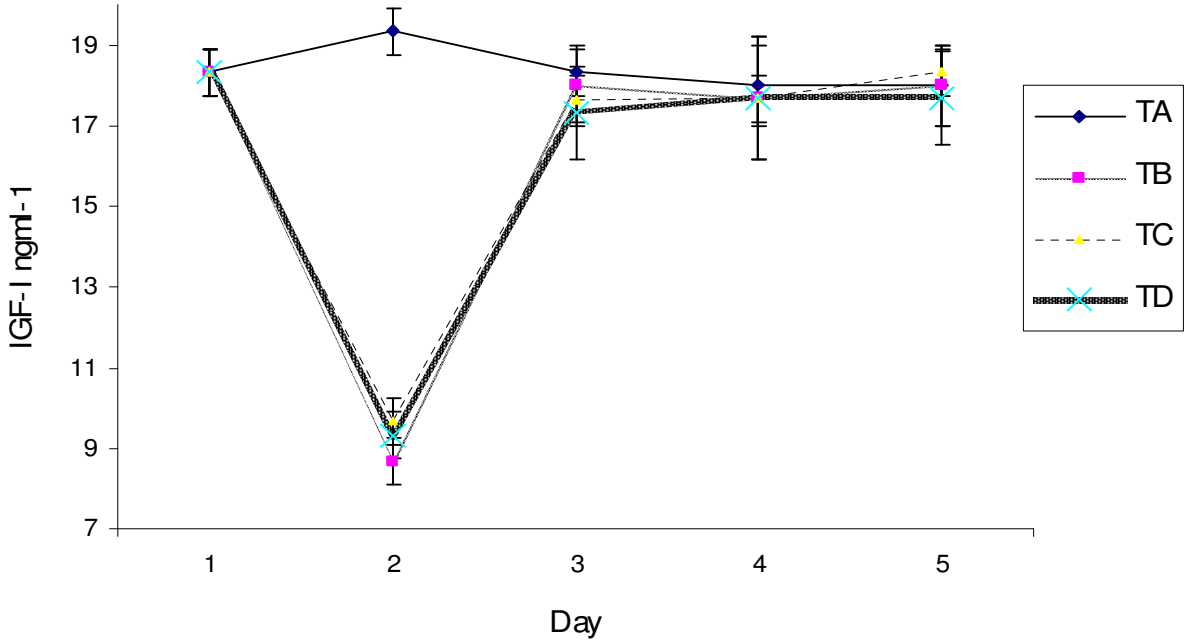

Figure 1. Plasma IGF-I change trends of different treatments during experiment.

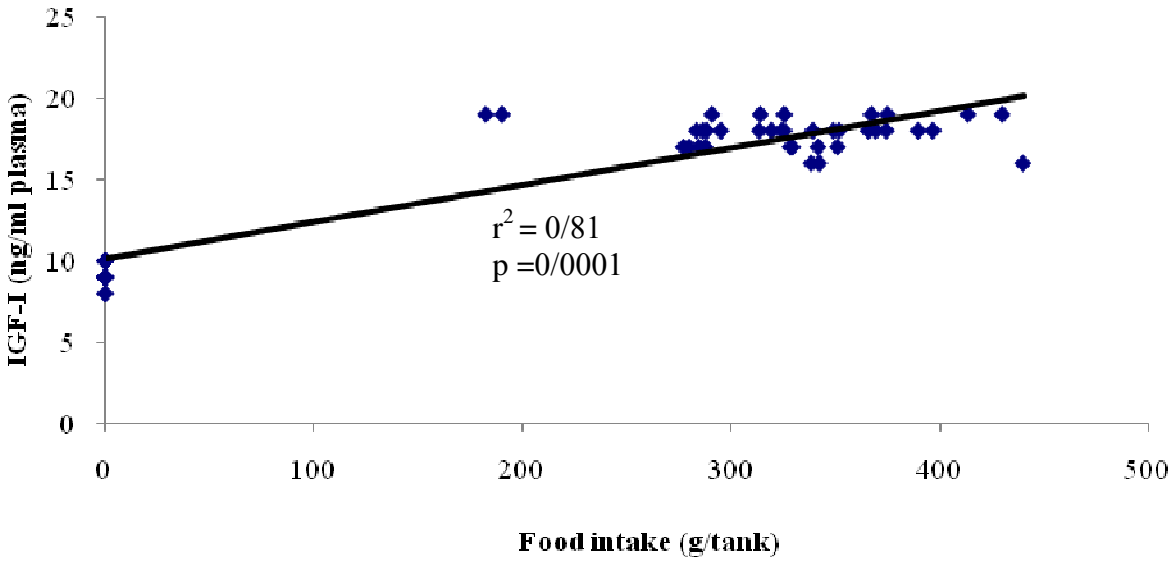

Figure 2. IGF-I and daily food intake correlation.

that could induce compensatory growth (CG). SGR of fasting groups confirm the findings of Quinton and Black (1990) who found that measures of growth such as SGR were significantly improved when rainbow trout fingerlings were re-fed, while some past studies including Weber and Bosworth (2005) achieved conflict results.

These differences in the findings could be due to starvation severity or experimental conditions. Fast growth ability after fasting periods could compensate for depressed growth in comparism to control group (Maclean and Metcalf, 2001; Xie et al., 2001; Zhu et al., 2001; Tian and Qin, 2004; Nikki et al., 2004). Higher SGR in re-feeding periods is one of compensatory growth indexes (Gaylord and GatlinIII, 2001; Nikki et al., 2004; Zhu et al., 2004). Therefore, higher SGR of TB and TC treatments have higher compensation in comparison to other groups.

Improved food coefficient could not be seen in all of the

groups. Results of food coefficient showed that feeding regimes did not improve but hyperphagia of B and C fish groups could be seen in comparison with control fishes. These results confirm some past studies (Gonzalez et al., 1995; Wang et al., 2005; Heide et al., 2006). Boujard et al. (2000) recorded reduced food coefficient and hyperphagia. Li et al. (2006) recorded decreasing of food conversion efficiency in fasting groups in comparison with control group. Probably, these differences could be due to differences in the experimental conditions, experimental design and physiological condition of animal (Jobling and Koskela, 1996). Hierarchy behavior could be seen in salmonids that reduce food coefficient (MCItyre et al., 1979; Jobling and Wansvike, 1983). Hierarchy due to increase in metabolical activities, energy consumption and decrease in food intake in dominant fish is because of their aggressive behaviors in feeding time; also decrease in food intake in other fishes is because of inhibiting actions of dominant fishes that reduce food coefficient (Jobling and Wandsvik, 1983). Causes of this problem could consist of hierarchy existence or experimental design and physiological conditions of fishes. Food intake results demonstrate that B and C groups have higher compensation in comparism to other groups. Results of SGR and food intake show TB, TC groups have higher compensation in comparism to D treatment. But B and C groups have no difference in comparism altogether.

IGF-I

The plasma IGF-I levels of the treatments, TB, TC and TD were significantly decreased during fasting and however, no significant differences were observed between the fasting groups. In contrast, the plasma IGF-I level in groups with refeeding started to increase gradually reaching the control level at the end of the re-feeding periods. Plasma IGF-I concentrations compensate in 12 initial days of refeeding periods then achieved permanent trend until end of refeeding periods. There were no differences between treatments. Effects of fasting and refeeding in mammalian demonstrated similar result like those in this study. This phenomenon has been observed in higher vertebrata like human, sheep and chick (Thissen et al., 1994). Changes of IGF-I during starvation and refeeding confirm some past studies (Duan and Hirano, 1992; Moriyama et al., 1994). Reduction of IGF-I concentration induces lipolysis and inhibits degradation of proteins (Perez-Sanchez and LeBail, 1999). Significant positive correlation exists between IGF-I and food intake (Figure 2) that demonstrates IGF-I effect by nutritional status. These results are confirmed by different studies in higher vertebrata (Thissen et al., 1994). Study on salmonids (Duguay et al., 1994), trout (Niu et al., 1993) and sea bream (Perez- sanchez et al., 1994) confirm synthesis and releasing of IGF-I, depending on nutritional status.

Several authors had suggested that IGF-I concentrations could be used to assess different diets and feeding regimes (Perez- Sanchez and LeBail, 1999; Dyer et al., 2004; Li et al., 2006). Past studies focus on diets assessment by physiological indicators including hormones, their receptors and else. Past studies on nutritional assessment by IGF-I were related to diets (Perez-Sanchez and LeBail, 1999; Dyer et al., 2004; Li et al., 2006). The major aim of present study was to answer this question: could IGF-I be used for CG assessment? IGF-I concentrations show no significant difference between treatments and control group at the end of experiment (Figure 1). These results showed that measurement of IGF-I could not be used as an index for assessment of CG. Studies of others on diets assessment conflict present result on CG assessment. These differences may refer to unclear knowledge and complication of CG phenomena or physiological conditions of fish and experiment conditions.

In conclusion, IGF-I could not be used for CG assessment alone. It should be used with other physiological elements such as other hormones, receptors and/or binding proteins that may be useful physiological tools for CG assessment in future studies.

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