EFFECT OF FASTING AND RE-FEEDING ON GROWTH PERFORMANCE, BIOCHEMICAL PARAMETERS OF BROWN TROUT (*SALMO TRUTTA FARIO)* AND RAINBOW TROUT (*ONCORHYNCHUS MYKISS)*

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ABSTRACT

The aim of this study was to investigate the effect of short-term fasting and re-feeding on growth performances, body compositions and biochemical parameters of brown trout (*Salmo trutta fario*) and rainbow trout (*Oncorhynchus mykiss*) with an average initial weight of 200±5 g. The 80 brown trout were placed in four tanks. 80 rainbow trout was placed in the other four tanks and 4 different feeding protocols were applied for both species; Control: every day, feeding twice a day; T1: 1 day of deprivation and3 days of re-feeding; T2: 2 days of deprivation and 6 days of re-feeding; T3: 3 days of deprivation and 9 days of re-feeding. Short-term fasting and re-feeding period took 30 days. At the end of the experiment, the weight gain (WG%) and specific growth rate (SGR%) of the control and treatment groups of the rainbow trout were higher than the values of the brown trout ($p<0.05$). But, no significant differences between the control and treatment groups of both species in terms of biochemical parameters and body compositions were observed ($p>0.05$). As a result, after the short-term fasting and re-feeding exposure, rainbow and brown trout showed a good growth performance. In addition, these trout could be fed without significant health damage in terms of physiological parameters.

Keywords: Rainbow trout, brown trout, growth, biochemical parameters, body composition, fasting, re-feeding.

INTRODUCTION

The aim of the implementation of feed protocols is to reduce the total production cost, to ensure economic sustainability and to reduce the amount of waste released the environment.Scientists working on fish feedinghave tried to find the most suitable feed and feeding patterns that may impact on feed intake and growthin the biology of the fish (Matilla *et al.,* 2009).In recent years, compensatory growth studies on various fish species have generally been done to determine growth performance, feed efficiency and nutrient uptake levels (Jobling and Koskela, 1996; Hayward *et al*., 1997; Oh *et al.,* 2007-2008; Wu *et al.,*2002; Ali and Jauncey, 2004; Zhu *et al.,*2005; Heide *et al.,*2006; Eroldogan *et al.,* 2008; Foss *et al.,*2009).Compensatory growthhas been classified in four different ways.These are full compensation, partial compensation, excessive compensation and the absence of compensation growth. The purpose of full compensation growth is that starved fish reach the same size as fed fish continuously. In partial compensation, starved fish do not reach the same size with continuously fed individuals. But, during the refeeding period, they show relatively good growth and feed conversion ratio (Hayward et al., 1997; Karatas, 2016). Overcompensation growth are seen when they reach a higher growth from the continuous feeding fish of starved fish. At the end of starvation period, if re-fed fish continue to grow up at an ordinary degree, in this case, it does not show any compensation (Ali *et al.,* 2003). Although deprivation–re-feeding cycles have been implemented to show compensatory growthin some fish species, there are no studies showing the effects of fasting and re-feeding on brown trout (Känkänen and Pirhonen, 2009).

The interest in compensatory growth in the aquaculture sector has led to an increase in the search for physiological responses of fish during fasting and refeeding. Studies conducted on mobilization of energy reserves against nutritional deprivation of fish may be helpful in determining the causes of the physiological responses (Yarmohammadi *et al.,* 2015). Liver enzymes, lipid profile and electrolyte levels may provide useful information about the physiological responses of fish (Bani &Vayghan, 2011). The aim of this study was to compare the impacts of short term fasting and refeeding on growth performances, body compositions and biochemical parameters of brown and rainbow trout.

MATERIALS AND METHODS

Animal material: Brown and rainbow trout having a weight of 200±5g was taken from Atatürk University, Faculty of Fisheries, Inland Water Fish Breeding and Research Center (13.06.2014). A total of 160 fish, 80 rainbow and 80 brown trout were divided into two groups and placed in two different tanks with capacity of approximately 700 L water. While one tank had only brown trout, the other tank had only rainbow trout. The tanks were filled with spring water with a constant flow (5 $L.min^{-1}$). The water flow was checked periodically and daily cleaning of the tanks were done (Karatas, 2016; Karatas, 2018a).

Experimental Design: After 14 days of adaptation period, 80 brown trout were taken from tank with capacity of approximately 700 L water were divided into 4 groups and randomly distributed to 4 tanks with 265 L water volume. 80 rainbow trout taken from other tank with capacity of 700 L waterwere divided into 4 groups and randomly distributed to 4 tanks with 265 L water volume and 4 different feeding protocols were applied for both species; Control: every day, feeding twice a day; T1: 1 day of deprivation and 3 days of re-feeding; T2: 2 days of deprivation and 6 days of re-feeding; T3: 3 days of deprivation and 9 days of re-feeding. Then, the fish were treated with 4 different feeding protocols (Eslamloo *et al.,* 2012); Control (fed two times daily); T1: Treatment 1 (1 days of fasting and 3 days of re-feeding); T2: Treatment 2 (2 days of fasting and 6 days of re-feeding) and T3: Treatment 3 (3 days of fasting and 9 days of re-feeding). Throughout assay, the rainbow and brown trout were weighed with precision scales every 10 days for 30 days and the daily feed amounts to be given to the fish at the end of the weighing were determined (Karatas, 2016). During the assay period, fish were fed with commercially available trout containing crude protein 45%, crude oil 20%, crude ash 10%, cellulose 3% and digestible energy 4000 kcal (Karatas, 2012).

Determination of growth rates: The weight gain (WG%), feed conversion ratio (FCR), feed efficiency rate (FER%) and specific growth rate (SGR%) in the control and treatment groups of the brown and rainbow trout were calculated by using the following equalities; weight gain (WG %) = 100[(Wt-W0)/ W0], feed conversion ratio (FCR) = intake (g, dry weight) / wet weight gain (g), specific growth rate (SGR) =100[(lnWt-lnW0)/t]; feed efficiency ratio (FER%) = 100. (wet weight gain (g) / intake (g)) (Azodi *et al.,*2013; Karatas, 2016).

Proximate analyses of tissues: Analyses of tissues were done by drying and homogenizing of the samples. The tissues were dried at 105°C for 5 h with the help of an oven in order to obtain a constant weight. Ash ingredient was detected by the combustion of tissue at approximately 550°C for 12 h in the ash oven, the protein ingredient of fish was detected with the Kjeldahl and Soxtec method (AOAC, 1995).

Water quality parameters: The water quality parameters in the work area were as follows, respectively; temperature (9.6-10.5^{0}C); pH (about 7.6±0.5); dissolved oxygen (about 10.6±0.60 mg/L); water hardness (about 175.1±3.21 mg/L) (Karatas *et al.,* 2014). No significant changes in water quality parameters during the experiment were observed.

Blood samples and serum: To reduce stress, the fish were caught very shortly and blood samples taken from the caudal vein by syringe were transferred to the anticoagulant tubes (Hedayati and Hosseini, 2013). Taken blood samples were incubated for 20 min to coagulate and then theywere centrifuged at 3000 rpm for 10 minutes and the serum was separated. Theserum samples were analyzed with autoanalyzer Cobas C501 using commercial kits (Karatas *et al.,* 2014).

Statistical Analyses: All results obtained from this study are expressed as mean ± STD. Data were analyzed with the aid of Statistical Package Programme (SPSS, ver. 20.0). Independent samples t test was applied to compare two independent groups. While repeated measures were used in more than two dependent group comparisons, Friedman test was used in those who did not show normal distribution. P<0.05 was thought to be statistically significant.

RESULTS

Throughout the entire assay, there was no death in the control and treatment groups of brown and rainbow trout. Results of the current study are indicated in Table 1, 2 and 3. At the end of the experiment, when the developmental parameters of control and treatment groups of brown and rainbow trout were evaluated separately, no significant difference was observed (p>0.05). However, SGR and WG values control and treatment groups of rainbow trout were higher than the values of brown trout(p<0.05). While moisture (%), ash (%), protein (%) ratios were observed increases in T1, T2 and T3 groups in both the brown and rainbow trout exposed to fasting and re-feeding periods compared to control groups, reductions in blood biochemical parameters of the groups (except for CI) were observed. However, no significant different between control and experiment groups in terms of growth performance (feed conversion ratio (FCR) and feed efficiency ratio (FER)), biochemical parameters (CHOL (Cholesterol), HDL (High Density Lipoprotein), LDL (Low Density Lipoprotein), TG (Triglyceride), Na (Sodium), Cl (Chlorine), Ca (Calcium), Mg (Magnesium), P (Phosphorus)and K (Potassium)) and body compositions (Moisture (%), Ash (%), Protein (%))levels were observed (p>0.05) (Table 1, 2 and 3).

DISCUSSION

The growth control is very important for aquaculture production. One of the most suitable methods for the growth control is the application of compensatory growth. Compensatory growth is very important for businesses engaged in aquaculture breeding such as feed utilization, increasing in growth rate, optimization and management of feed, minimizing feed wastage. In the present study, the specific growth rate (%) and weight gain (%) among the treatment and control groups of both species showed a tendency to decrease with increasing feed deprivation ($p>0.05$) (Table 1). However, the SGR and WG values of the control and treatment groups of the rainbow trout were significantly higher than the values of the brown trout.

Table 1: Growth performance values of brown and rainbow trout reared at four feeding regimes C: Control (fed two times daily); T1: Treatment 1 (1 days of starvation and 3 days of re-feeding); T2: Treatment 2 (2 days of starvation and 6 days of re-feeding) and T3: Treatment 3 (3 days of starvation and 9 days of re-feeding).

	Brown trout				Rainbow trout			
Indices	**C**	**T1**	**T2**	**T3**	**C**	**T1**	**T2**	**T3**
WG (%)	45.1±2.10 ^{a}	42.5±2.09 ^{a}	40.0±3.05 ^{a}	35.2±2.71 ^{a}	97.0±5.8^b	94.7±8.1 ^{b}	92.5±6.0 ^{b}	90.5±7.2 ^{b}
SGR (% /day)	1.26±0.09 ^{a}	1.20±0.05 ^{a}	1.16±0.07 ^{a}	1.13±0.03 ^{a}	2.26±0.02 ^{b}	2.23±0.09 ^{b}	2.20±0.05 ^{b}	2.16±0.02 ^{b}
FCR	1.14±0.07	1.15±0.02	1.12±0.04	1.10±0.06	1.12±0.07	1.10±0.02	1.09±0.06	1.08±0.04
FER(%)	88.5±4.03	87.0±3.08	89.3±5.05	91.3±3.62	89.3±3.05	91.3±4.21	92.1±3.2	92.5.±2.01

Results were given as mean±SD. SGR (specific growth rate) $=100[(\ln W_t-\ln W_0)/t]$; WG (weight gain) $= 100[(W_t-W_0)/ W_0]$. FER (feed efficiency ratio) = 100.(wet weight gain (g) / intake (g)). FCR (feed conversion ratio) = intake (g, dry weight) / wet weight gain (g). Different letters indicate differences between groups

Table 2: Changes in the body composition of brown and rainbow trout reared at four feeding regimes C: Control (fed two times daily); T1: Treatment 1 (1 days of starvation and 3 days of re-feeding); T2: Treatment 2 (2 days of starvation and 6 days of re-feeding) and T3: Treatment 3 (3 days of starvation and 9 days of re-feeding).

	Brown trout				Rainbow trout			
Indices	**C**	**T1**	**T2**	**T3**	**C**	**T1**	**T2**	**T3**
Moisture (%)	65.5±3.15	65.6±4.26	65.7±4.18	66.1±5.23	65.9±4.82	66.1±5.16	66.4±4.35	66.8±5.13
Ash (%)	1.45±0.18	1.50±0.05	1.54±0.04	1.60±0.08	1.48±0.12	1.52±0.14	1.55±0.14	1.62±0.28
Protein (%)	17.8±1.82	17.9±1.21	18.2±2.25	18.4±1.47	18.1±2.35	18.2.±2.64	18.5±1.97	18.7±2.47

No significant difference was observed between control and the treatments group of brown trout and rainbow trout.

Table 3: Changes in liver function tests, lipid profiles, electrolyte levels of fasting and re-fed in brown and rainbow trout

	Brown trout				Rainbow trout			
				Lipid profile				
Indices	**Control**	**T1**	**T2**	**T3**	**Control**	**T1**	**T2**	**T3**
CHOL	296.8 ±63.8	295.6 ±76.2	284.2 ±43.7	272.2 ±40.5	312.5±45.6	310.2 ±35.4	294.1 ±21.4	291.4±25.7
HDL	197.8 ±40.1	193.0±18.4	189.2 ±23.0	188.0±30.6	201.7 ±47.9	197.5 ±40.6	195.7 ±49.4	195.0±54.5
LDL	124.2 ±26.4	121.2 ±27.8	116.6 ±25.6	113.5 ±22.0	126±34.9	123.0±34.1	121.7 ±35.2	118.7 ±34
TG	397.4 ±42.7	392.2 ±32.8	387.4±35.7	380.4 ±36.0	401±32.1	396.7 ±35.9	391.7±39.8	375±32.7
				Liver Function Tests				
AST	521.2±52.6	516.2±59.9	510.7 ±40.3	508.5 ±42.1	543.4 ±45.2	533.6 ±56.0	557.8 ±46.0	553.2 ±56.9
ALT	26.8 ±3.2	26.0±7.3	25.8±5.9	24.6 ±4.2	26.1±4.5	25.5 ±5.9	24.4±5.1	23.5 ±6.2
				Electrolytes				
Ca^{++}	11.7±3.7	11.6±2.4	10.5±2.7	10.1±2.3	11.2±1.50	11.1 ±0.65	10.9±1.09	10.7 ±1.33
Mg^{++}	3.1±0.2	3.0±0.2	2.9±0.1	2.9±0.2	4.3 ±0.77	3.9 ±0.68	3.8 ±1.30	3.7 ±0.40

Na^+	156±1.8	155.2±2.7	154.4±1.5	154.6±3.2	157 ±1.4	156.5 ±2.08	154.7 ±1.70	153.7 ±2.5
K^+	1.0±0.1	0.95 ±0.4	0.94±0.3	0.94±0.3	0.90 ±0.11	0.88 ±0.08	0.85±0.10	0.86 ±0.09
Cl^-	131.8±1.9	131.0 ±5.3	135.2±0.8	135.2±3.7	129.5±4.4	130.5 ±5.4	132.5 ±4.0	134.5 ±2.5
P^-	13.1±5.2	12.8±2.4	12.5±3.2	12.6±2.4	13.5 ±5.8	13.1 ±4.3	13.0 ±3.2	12.9 ±3.5

No significant difference was observed between control and the treatments groups of brown trout and rainbow trout.

This may be due to the reduced metabolic rate during feed deprivation as well as decreased growth and daily feed intake or stress (Heide *et al.,* 2006). The results obtained in this study were comparable with the reported results in European minnows by Russel and Wootton (1992); in rainbow trout with different periods of starvation and refeeding by Weatherly and Gill (1981), Dobson and Holmes (1984), Quinton and Blake (1990), Nikki *et al.* (2004), in Atlantic cod by Jobling *et al.* (1994); in pikeperch by Mattila *et al.* (2009); Chinese sturgeon by Liu *et al.* (2011) and Xie *et al.* (2001). Partial compensation was reported in Atlantic charr by Jobling *et al.* (1993), in gilthead sea bream by Eroldogan *et al.* (2006), Atlantic halibut by Heide *et al.* (2006) and in White fish by Känkänen and Pirhonen (2009). However, the results of this study were different from the studies showing complete compensation growth as reported incommon carp by Schwarz *et al.* (1985), great sturgeon by Falahatkar *et al.* (2009), gilthead sea breams by Bavcevic *et al.* (2010), sea breams by Peres *et al.* (2011).

FER and FCR values of control group and rainbow trout were higher than those of control and starved brown trout. (Table 1). But, the difference was statistically non-significance($p>0.05$) (Table 1). It may be due to the slow digestion in the brown trout when compared to rainbow trout. The results obtained from brown and rainbow trout were different from results reported by Tian and Qin (2003), Azodi *et al.* (2013) Azodi *et al.* (2015) and Karatas (2018a). Different in the FER and FCR values may be related to fish size and feed patterns.

The body compositions of the rainbow and brown trout subjected to fasting was similar to control groups. In other words, there was non-significant effect on body moisture and ash of short-term fasting and re-feeding applied to brown and rainbow trout ($p>0.05$) (Table 2). This is in accordance with the results on rainbow trout (Quinton and Blake, 1990; Karatas, 2018a), barramundi (Tian and Qin 2003; Tian and Qin, 2004) and gilthead sea (Eroldogan *et al.,*2008). But, were in conflict with the results of Wang *et al.* (2000) and Matilla *et al.* (2009). No differences in the protein content of rainbow and brown trout exposed to short-term fasting and re-feeding periods were observed ($p>0.05$) (Table 2). But, there were increases. The results of this study were in accordance with those of Azodi et al. (2013), Tian and Qin (2004) and Karatas (2018a). But, the results of studies reported by Quinton and Blake (1990), Xie *et al.* (2001), Tian and Qin (2003), Iqbal et al. (2006) and Matilla *et al.* (2009) were different from the results of this study.

Physiological parameters are used to determine the responses of fish to environmental conditions, malnutrition and harmful substances (Rehulka, 2000). It has been reported that the liver is the first organ affected by nutrient deficiency in living things (Navarro and Gutiérrez, 1995; Power *et al.,* 2000). In this study, there was no significant change ($p>0.05$) in liver enzyme (AST and ALT) levels of the fasting and re-fed groups of brown and rainbow trout when compared with the control groups (Table 3). The minimum and maximum values of AST and ALT were observed in the range of 366.0-573.0 and 8.8-30.2 mg/dL, respectively (Karatas, 2012). The results of this study were consistent with the results of Karatas (2012). It was showed that the feeding protocols applied for this study did not cause any damage to the liver. The results obtained in brown and rainbow trout were in accordance with the results of (Azodi *et al.,* 2015).

Lipids have been reported to be effective in rising the release of fatty acids from adipose tissue and liver during deprivation in most species of fish (Azodi *et al.,* 2015). Lipids, which form the long-term energy reserves of a living organism, are usually transported in the form of lipoprotein compounds (Pamela *et al.,*2004). Triglycerides, which are energy reserves, are effective in short-term fasting periods (Navarro and Gutiérrez, 1995). In this study, there was no significant change ($p>0.05$) in the lipid profiles of brown and rainbow trout exposed to periods of fasting and re-feeding (Table 3). But, there were decreases. The minimum and maximum values of cholesterol and triglycerides in blood lipids were observed in the range of 0.10-714.29 mg/dL, 0.40-999.00 mg/dL, respectively (Çelik and Bilgin, 2007). The results of this study were consistent with the results of Çelik and Bilgin (2006). Studies on different fish species have reported no significant change in CHOL, HDL, LDL and TG levels (Azodi *et al.,* 2015; Furne´ *et al.,* 2012; Pérez-Jiménez *et al.,* 2012; Pérez-Jiménez *et al.,* 2007). Whereas, some studies showed that there might be a decrease in TG levels of some fish during fasting (Costas *et al.,* 2011; Falahatkar, 2012; Karatas, 2018b). Nutrition is one of the most important factors in meeting the mineral needs of fish (Karagül *et al.,* 2000). If minerals are taken in inadequate levels, they cause many vital functions in the body to deteriorate (Johannessen and Dahl, 1996; Conte, 2004). Minerals such as Na^{+}, CI^{-} and $K^{+}$are found in the extracellular and intracellular fluid. The main cation of extracellular fluid is sodium and the main anion is chloride. K^{+} and$Mg^{+2}$are known as the main cation of intracellular fluid. Na^{+}, CI^{-} and K^{+} are important for maintaining homeostasis (Terry, 1994).The Mg^{+2} ion is also an important mineral in neurochemical transmission and muscle stimulation (Karnaky,1998). Ca^{++} is involved in blood clotting as well as blood pressure regulation (Junge, 2006). Phosphorus is involved in the synthesis of proteins, enzymes and energy production in the cell.In the present study, there was no statistically significant difference between Ca, Na, P, Cl and Mg values ofbrown and rainbow trout exposed to periods of fasting and re-feeding($p>0.05$). The minimum and maximum values of sodium, potassium, calcium, magnesium, chlorine and phosphorus from blood electrolytes are in the range of 61.8-264.8, 0.6-14.3, 0.2-11.0, 0.04-4.6, 73.0-196.0 and 1.33-7.98 $mmoll^{-1}$, respectively (Çelik, 2006). The results of this study were consistent with the results of Çelik (2006). Lyytikainena *et al.* (2002) and Keleştemur *et al.* (2012) determined that there was no statistically significant difference in calcium, sodium and chloride concentrations under stress, fluctuating and constant thermal conditions. It has been reported that the stress caused by starvation and malnutrition leads to a decrease in the potassium concentration of fish (Lyytikainena *et al.,* 2002; Keleştemur *et al.,* 2012).

Conclusion: The results of this study showed that 1-3 days fasting and re-feeding programs can be a good feed management tool for rainbow and brown trout. These trout could be fed without significant health damage in terms of physiological parameters. Further research should be carried on different feeding models for compensatory growth of brown trout, *Salmo trutta fario*.

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