



Effect of Short-Term Starvation on Serum Metabolites, Antioxidant Enzymes and Endogenous Reserves of Rainbow Trout, *Oncorhynchus mykiss*

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ABSTRACT

This study was conducted to investigate the effects of starvation for 8 days on the serum metabolites and antioxidant enzymes, thiobarbituric acid reacting substance levels, endogenous reserves in liver and muscle tissues of rainbow trout (*Oncorhynchus mykiss*). Eight-days of fasting caused a significant decrease in glucose, total protein, triglyceride, cholesterol, high-density lipoprotein and low-density lipoprotein levels as well as protein and lipid reserves in liver and muscle tissue of fish ($p < 0.05$). The fasting period had no significant effect on the hepatic thiobarbituric acid reactive substances (TBARS), but, it caused a significant decrease in the hepatic catalase and glutathione peroxidase ($p < 0.05$). In muscle tissue, the TBARS levels were significantly decreased ($p < 0.05$). Whereas, antioxidant enzyme activities remained unchanged ($p > 0.05$). The results obtained from this study showed that the short-term starvation leads to significant changes on serum metabolites, antioxidant enzyme activities and endogenous reserves of rainbow trout.

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Key words

Rainbow trout, Fasting, Metabolic effect, Lipid peroxidation, Antioxidant enzyme.

INTRODUCTION

Many fish species have the ability to tolerate starvation because they cannot adequately meet their nutritional needs as well as breeding and migration (Hinch *et al.*, 2005; Miller *et al.*, 2009). The use of energy in fish varies depending on particularly adverse environmental conditions and food insufficiency (Salem *et al.*, 2007). If the fish cannot feed adequately, activate their endogenous reserves to maintain their vital processes such as brain function, regulation of respiration and mineral balance (Furne *et al.*, 2012). Endogenous reserves are generally regarded as a reflection of the metabolic responses of fish to environmental conditions and malnutrition (Wang *et al.*, 2006; Furne *et al.*, 2012). These are also due to different age, size and being natural or cultured fish (Navarro and Gutierrez, 1995). In some fish, liver glycogen is first used during fasting (Hung *et al.*, 1997; Figueiredo-Garutti *et al.*, 2002; Meto'n *et al.*, 2003). During the first stages of a starvation, the hydrolysis of glycogen has a significant effect in the regulation of blood glucose homeostasis that is to deliver glucose to blood after glycogenolysis from glycogen in liver at short-term starvation (Furne *et al.*, 2012). In other words, glucose homeostasis is the

regulation of the concentration of glucose. In addition, fat reserves are used as a last metabolite, if the starvation is increased a great degree. However, studies made on some species of fish have reported that fish use energy from the muscle protein of the last active reserve if the available energy sources are not sufficient (Navarro and Gutierrez, 1995). Conversely, studies on some species have reported that liver glycogen deposits try to regulate reduced protein and lipid levels for glyconeogenesis (Sheridan and Mommsen, 1991; Navarro and Gutierrez, 1995; Gillis and Ballantyne, 1996; Furne *et al.*, 2012).

The fish are generally exposed to two types of starvation. These are short term and long-term starvation. Short-term nutritional starvation accelerates aging, injury, toxicity of chemicals and diseases in fish (Pascual *et al.*, 2003; Najafi *et al.*, 2014). Also, short-term nutritional starvation causes the reduction of antioxidant deposits in the organs of organisms and increased production of free radicals in liver (Pascual *et al.*, 2003; Furne *et al.*, 2009). This process can be attributed to increase of reactive oxygen species generated by starvation and leading to oxidative stresses (Robinson *et al.*, 1997). Oxidative stress is a process related to the inadequate removal of reactive oxygen species from where it is present (Sies, 1986). Therefore, organisms are protected from reactive oxygen species by means of different defense mechanisms (Pascual *et al.*, 2003). The defense mechanisms are catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR). Antioxidants with low molecular weight such as glutathione, E and C

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vitamins act by enzymatic defense mechanisms (Furne *et al.*, 2009, 2012).

The aim of this study was to determine alterations in levels of serum metabolit (Glucose, total protein, triglyceride, cholesterol, HDL, LDL), antioxidant enzymes activities such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR), as well as thiobarbituric acid reacting substances (TBARS) levels, a lipid peroxidation product, in the liver and muscle tissues and endogenous reserves such as protein and lipid of rainbow trout.

MATERIALS AND METHODS

Experimental animals

120 rainbow trout (*Oncorhynchus mykiss*) average weight 170 g were obtained from the fish farm, 41 km away from Ağrı province. A group of 40 fish were divided into four groups, each of ten. One was fed twice a day (Control), while the others were starved for a day (T1), 2 days (T2) and 8 days (T3). This experiment was repeated three times. The temperature, dissolved oxygen and pH of the water in which the fish was about 12±0.8°C, 10.3 and 7.5, respectively.

Biochemical analysis of blood, liver and muscle

Blood samples were taken from the caudal veins of the fish with the help of a 5 cc syringe and transferred to anticoagulation tubes and used for the estimation levels of glucose, total protein, triglyceride, cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) according to Karataş and Kocaman (2014). Briefly, the blood samples taken from fish were centrifuged at 3000 rpm for 10 minutes and the serum was separated. Glucose, total protein, triglyceride, cholesterol, HDL, LDL and triglyceride were analyzed with autoanalyzer Cobas C6000 using commercial biochemical kits. The samples of liver and muscle tissues were placed in liquid nitrogen and stored at -80°C until analyzed according to the method of Furne *et al.* (2012).

Measurement of TBARS and antioxidant enzyme activities

TBARS levels, a lipid peroxidation product, as well as antioxidant enzymes activities such as CAT (EC 1.11.1.6), SOD (EC 1.15.1.1), GPx (EC 1.11.1.9) and GR (EC 1.8.1.7) were analysed. Antioxidant enzyme activities in liver and muscle tissues (SOD, CAT, GPx and GR and TBARS levels were measured according to the methods defined by Furne *et al.* (2012).

Statistical analysis

Data were analyzed by SPSS 20.0 for Windows, using one-way analyses of variance (ANOVA). Differences were determined by using Duncan's multiple range tests. P<0.05 was considered statistically significant.

RESULTS

The data obtained from this study and the statistical analyzes of these data are summarized in Tables I, II and III. According to these results, levels of blood glucose, triglyceride, cholesterol, HDL and LDL of T3 group were significantly decreased compared to other groups (p<0.05) (Table I). Starvation had no significant effect on blood total protein levels and liver lipid reserves of all the three starvation groups when compared to control group (Table II). However, liver protein reserves of T3 group (p<0.05) were significantly reduced when compared to other groups. Protein and lipid reserves in muscle tissue decreased significantly only in the T3 group (p<0.05) compared to the other groups (Table II). In liver tissues of groups (Table III), although there were slight fluctuations in thiobarbituric acid reactive substances (TBARS) levels of all the three starvation groups compared to control group (p>0.05), significant decreases in levels of CAT and GPx of T3 group were observed (p<0.05). No significant difference levels of SOD and GR were observed (p>0.05). In muscle tissues, while TBARS level in T3 group were significantly decreased (p<0.05), SOD, CAT, GPx and GR levels were increased. However, these increases were not statistically significant (p>0.05) (Table III).

Table I.- Changes in the serum metabolites of rainbow trout during short-term fasting.

Metabolites (mg dL ⁻¹)	Control	T1	T2	T3
Glucose	93.6±2.65 ^a	91.2±2.41 ^a	79.3±1.20 ^a	56.3±2.58 ^b
Total protein	3.60±0.25 ^a	3.58±0.32 ^a	3.54±0.34 ^a	3.49±0.27 ^a
Total cholesterol	319.1±4.13 ^a	319.8±5.17 ^a	314.5±7.10 ^a	210.2±3.24 ^b
HDL	101.4±3.90 ^a	100.4±3.80 ^a	94.2±3.70 ^a	73.6±2.13 ^b
LDL	180±7.9 ^a	182±3.08 ^a	191.6±8.56 ^a	90.3±10.40 ^b
Triglycerides	395.6±14.3 ^a	391.6±18.54 ^a	297.3±12.23 ^a	201.4±9.51 ^b

The results were given as mean and standard deviation. Different letters indicate differences between groups. One was fed twice a day (Control), a day (T1), 2 days (T2) and 8 days (T3) of starvation.

Table II.- Alterations in levels of protein and lipid in liver and muscle tissues of rainbow trout during short-term fasting.

% wet weight	Tissues	Control	T1	T2	T3
Protein	Liver	11.34±1.76 ^a	11.41±2.14 ^a	10.96±2.25 ^a	8.75±0.35 ^b
Protein	Muscle	7.15±0.47 ^a	6.94±0.55 ^a	6.53±0.51 ^a	3.27±0.19 ^b
Lipid	Liver	4.86±0.26 ^a	4.83±0.39 ^a	4.45±0.41 ^a	4.16±0.37 ^a
Lipid	Muscle	18.91±2.21 ^a	18.63±2.19 ^a	14.71±0.89 ^a	8.75±2.14 ^b

The results were given as mean and standard deviation. Different letters indicate differences between groups. One was fed twice a day (Control), a day (T1), 2 days (T2) and 8 days (T3) of starvation.

Table III.- Alterations in thiobarbituric acid reactive substances (TBARS) levels and the activities of catalase (CAT) glutathione reductase (GR) glutathione peroxidase (GPx) and superoxide dismutase (SOD) in liver and muscle of rainbow trout during short-term fasting.

Lipid peroxidation and antioxidant enzymes	Control	T1	T2	T3
Liver				
TBARS (nmol g ⁻¹ tissue)	40.3±2.57 ^a	41.2±2.47 ^a	44.3±2.03 ^a	48.7±2.04 ^a
CAT (U mg ⁻¹ protein)	358.1±10.3 ^a	355.4±8.71 ^a	341.5±8.17 ^a	337.2±5.49 ^b
SOD (U mg ⁻¹ protein)	635.3±20.4 ^a	630.1±6.53 ^a	614.2±6.15 ^{ab}	588.0±7.6 ^a
GPx (mU mg ⁻¹ protein)	483.2±19.6 ^a	485.1±16.2 ^a	455±30.4 ^{ab}	403.7±41.4 ^b
GR (mU mg ⁻¹ protein)	15.4±2.86 ^a	15.8±3.05 ^a	14.7±1.90 ^a	14.3±2.11 ^a
Muscle				
TBARS (nmol g ⁻¹ tissue)	80.4±5.8 ^a	80.1±4.7 ^a	77.3±4.6 ^a	60.5±1.2 ^b
CAT (U mg ⁻¹ protein)	0.034±0.02 ^a	0.036±0.10 ^a	0.046±0.02 ^a	0.058±0.02 ^a
SOD (U mg ⁻¹ protein)	62.4±1.8 ^a	63.1±3.1 ^a	68.7±2.27 ^a	71.5±1.3 ^a
GPx (mU mg ⁻¹ protein)	139.2±10.3 ^a	141.2±10.3 ^a	149.8±11.5 ^a	155.2±20.8 ^a
GR (mU mg ⁻¹ protein)	4.21±1.5 ^a	4.60±1.02 ^a	4.90±0.71 ^a	5.12±1.9 ^a

The results were given as mean and standard deviation. Different letters indicate differences between groups. One was fed twice a day (Control), a day (T1), 2 days (T2) and 8 days (T3) of starvation.

DISCUSSION

The physiological process is considered as a reflection on fish alterations in environmental conditions such as temperature, hardness, pH *etc.* and harmful substances including oxidative stress and fasting stress. Fasting stress in fish can be affected by many factors such as fasting time, species of fish, and metabolism. These factors may be one of the most important reasons for the change in serum metabolite levels of fish. Fasting can be used to show species-specific differences in the metabolism and regulation of blood glucose (Kim *et al.*, 2014). In this study, glucose level decreased significantly in the T3 group when compared to the other groups. Kim *et al.* (2014) reported a decrease in plasma glucose levels of the salmonids such as brook trout, *S. fontinalis* (Heming and Paleczny, 1987), Chinook salmon, *O. tshawytscha* (Barton

et al., 1988) and Rainbow trout, *O. mykiss* (Farbridge and Leatherland, 1992). On the other hand, there are studies indicating an increase in plasma glucose levels of fish such as, jundia, *Rhamdia quelen* (Barcellos *et al.*, 2010), *Dicentrarchus labrax*, European seabass (Chatzifotis *et al.*, 2011), Senegalese sole, *Solea senegalensis* (Costas *et al.*, 2011) or red porgy (Caruso *et al.*, 2010), Antarctic fish, *Notothenia coriiceps* (Stepanowska *et al.*, 2006). But, no significant increases were reported (Kim *et al.*, 2014; Shabana *et al.*, 2017). These results were consistent with previous studies (Heming and Paleczny, 1987; Barton *et al.*, 1988; Farbridge and Leatherland, 1992). Kim *et al.* (2014) reported fasting in many fish species including European eel (Dave *et al.*, 1975), brook trout (Heming and Paleczny, 1987) and Senegal sole (Costas *et al.*, 2011) caused significant reductions in plasma protein concentration levels. In this study, protein levels in liver

and muscle tissues of T3 group was significantly reduced when compared to other groups. Decrease in the protein reserves of the fish during fasting period may be associated with converting energy of protein via gluconeogenesis. [Cho \(2009\)](#) reported that protein levels can be a good indicator in determining the severity of fasting. Another reason for the decrease in protein levels in the liver and muscle tissues may be due to either reduction in ribosomes or slowing of protein synthesis depending on starvation. These results were consistent with studies made by [Dave et al. \(1975\)](#), [Heming and Paleczny \(1987\)](#), [Costas et al. \(2011\)](#) and [Cho \(2009\)](#).

Cholesterol is needed in the synthesis of sexual hormones and for cell membranes ([Kulkarni and Barad, 2015](#)). In this study, level of total cholesterol of T3 group was statistically decreased significant compared to other groups. [Furne et al. \(2012\)](#) reported that the rainbow trout exposed to 10-day hunger had significant reductions in total cholesterol levels. In another study, [Black and Skinner \(1986\)](#) reported that there were no significant effects on levels of serum cholesterol of the rainbow trout of fasting. Results obtained in this study groups were similar with results of [Black and Skinner \(1986\)](#) and [Furne et al. \(2012\)](#).

Triglycerides, responsible for providing the energy and nutrient needs of the cells, are known deposits of fat in the body ([Kulkarni and Barad, 2015](#)). In this study, there was a statistically significant decrease in the triglyceride ratio of the T3 group. This condition may be associated with decrease in the phospholipids of the T3 group since blood serum in the fish is an important lipid carrier. These results were consistent with those of [Friedrich and Stepanowska \(2001\)](#) and [Kulkarni and Barad \(2015\)](#).

Lipoproteins are transported into the body fluid in the form of complexes with proteins of water-insoluble lipids. Lipids, triglycerides, cholesterol esters, free cholesterol and phospholipids contain one or several different protein molecules. Lipoproteins have two important groups: LDL and HDL. HDL is rich in phospholipids and cholesterol. While HDL is carried from the peripheral tissues to liver to lipid metabolites, LDL carries lipid components from the tissues around the liver ([Zech et al., 1986](#); [Karatas and Kocaman, 2012](#); [Karatas and Kocaman, 2014](#)). In this study, levels of HDL and LDL of T3 group were significantly decreased compared to the other groups. Decreases in HDL and LDL levels may be associated with a decrease in total cholesterol levels. These results were consistent with the results of [Kulkarni and Barad \(2015\)](#).

The use of hepatic lipids as an energy source during fasting varies according to species and lipid reserve tissue ([Godin and Wohaieb, 1988](#)). The energy that the body needs is usually provided by activating other reserves,

such as carbohydrates ([Kim et al., 2014](#)). In this study, lipid levels were significantly decreased in T3 group. The decrease in muscle lipid levels in fish may be indicative of the use of lipid reserves for energy purposes or during fasting of fish may be associated with decreased lipid mobilization between the liver and perivascular tissues. In this sense, decreases in HDL and LDL levels may be responsible for slowing or decreasing of lipid synthesis.

It was determined that the environmental conditions altered the level of lipid peroxidation and antioxidant enzymes ([Drozd et al., 2014](#); [Panicz et al., 2017](#)). The formation of oxidative stress in fish is usually associated with an increase in the level of lipid peroxidation by the effect of starvation and starvation stress ([Furne et al., 2009](#)). Some authors working on different fish species reported that starvation and starvation stress increased the level of lipid peroxidation in liver of fish such as rainbow trout (*Oncorhynchus mykiss*), gilthead seabream (*S. aurata*) and common dentex (*D. dentex*) ([Hidalgo et al., 2002](#); [Pascual et al., 2003](#); [Morales et al., 2004](#)). It was reported that carp applied diets increased lipid peroxidation and decreased antioxidant level ([Stepanowska and Sawicka, 2006](#)). Regarding the activities of liver antioxidant enzymes of rainbow trout, in this study, there was a decrease in antioxidant enzymes such as CAT and GPx due to the increase in fasting time. These decreases are due to a decrease in enzyme synthesis substrates, possibly may cause an increase in lipid peroxidation levels. These findings were consistent with the results of [Furne et al. \(2009\)](#) and [Godin and Wohaieb \(1988\)](#). [Blom et al. \(2000\)](#) and [Morales et al. \(2004\)](#) have been reported that starvation is caused by the decrease in GR enzyme activity of rainbow trout and common dentex. [Pascual et al. \(2003\)](#) reported a reduction in CAT and GPx levels while SOD and GR levels were elevated in fasting conditions. [Morales et al. \(2004\)](#) reported that there is a significant effect of deprivation on SOD, CAT and GPx levels. Therefore, the liver is probably considered one of the best parameters to demonstrate antioxidant defenses in organisms ([Chance et al., 1979](#); [Davies, 1991](#); [Wilhelm-Filho et al., 1993](#)). In contrast to liver tissue, level of TBARS in muscle tissue of T3 group was significantly reduced. Decreased TBARS levels are likely due to the increase in activities. Another reason for the decrease in TBARS levels may be due to a reduction in energy reserves in muscle tissue. Because, the fish in fasting state may activate the energy reserves in muscle tissue and thus may decrease the effects of lipid peroxidation (TBARS).

CONCLUSION

Results obtained in this study showed that short-

term fasting was caused significant changes on the serum metabolit levels, endogenous reserves such as protein and lipid and antioxidant enzymes activities of rainbow trout. Therefore, serum or plasma metabolites, liver and muscle tissues may be important in determining the effects on different fish species of starvation. Further studies are needed to determine the effects of starvation on fish physiology.

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Statement of conflict of interest

No conflict of interest was reported by the authors.

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