

Comparison of Paraoxonase Activity, Malondialdehyde and High-Density Lipoprotein Levels in Cultivated Normal and Albino Rainbow Trout Reared in the Same Conditions

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Makale Kodu (Article Code): KVFD-2011-4971

Summary

In this study, serum paraoxonase activity (PON), malondialdehyde (MDA) and high-density lipoprotein (HDL) levels of culture rainbow trout (NRT) and albino rainbow trout (ART) have been compared. In this research, ART and NRT which have average weights of $130\pm 10.7g$ and $150\pm 11.9g$ respectively have been used. During the research, fish (ART and NRT) have been fed with commercial trout feed, twice a day. Fish used in the analysis have been random-ly caught from pools and five fish (three times replicated) for each group have been sampled. PON activity, HDL and PON/HDL levels determined in serum samples of NRT group have been found higher than the ART group. Mean MDA level in NRT group has been found lower than the ART group, however, these differences have been found statistically insignificant. Finally, the different species and size, dominance status may be reason for the change of PON activity, MDA and HDL levels.

Keywords: Rainbow trout, Albino rainbow trout, Malondialdehyde, Paraoxonase activity, High density lipoprotein

Aynı Koşullarda Yetiştirilen Normal ve Albino Gökkuşığı Alabalıklarının Paraoksonaz Aktivitesi, Malondialdehit ve Yüksek Yoğunluklu Lipoprotein Seviyelerinin Karşılaştırılması

Özet

Bu çalışmada, aynı koşullarda yetiştirilen kültür gökkuşığı alabalığı (NRT) ve albino gökkuşığı alabalığının (ART) serum paraoksonaz aktivitesi (PON), malondialdehit (MDA) ve yüksek yoğunluklu lipoprotein (HDL) seviyeleri karşılaştırılmıştır. Araştırmada, ortalama ağırlığı $150\pm 11.9g$ olan normal gökkuşığı alabalıkları ile $130\pm 10.7g$ olan albino gökkuşığı alabalıkları kullanılmıştır. Araştırma süresince, balıklar günde iki kez ticari alabalık yemiyle beslenmiştir. Analizlerde kullanılan balıklar havuzlardan rasgele yakalanmış ve her grup için beş balık (üç tekrarlı) örneklenmiştir. NRT'dan elde edilen serum örneklerinde belirlenen PON, HDL ve PON/HDL seviyeleri ART'a göre yüksek bulunmuştur. NRT'da ortalama MDA seviyesi ART'a göre düşük bulunmuş ancak bu farklar istatistik olarak anlamlı bulunmamıştır. Sonuç olarak, tür farklılığı, büyüklük ve baskınlık durumu PON aktivitesi, MDA ve HDL seviyelerinin değişmesinin sebebi olabilir.

Anahtar sözcükler: Gökkuşığı alabalığı, Albino gökkuşığı alabalığı, Malondialdehit, Paraoksonaz aktivitesi, Yüksek yoğunluklu lipoprotein

INTRODUCTION

Oxidative metabolism of cells is source of reactive oxygen species (ROS), resulting from reduction of oxygen, that can damage most cellular components leading to cell death. To protect from these highly reactive intermediates, living organisms have a defense system both enzymatic

and non-enzymatic antioxidants ¹. MDA is a lipid peroxidation product and it is the most frequently used as biomarker for assessing in vivo oxidative stress. In the other words, MDA is a marker of oxidant/antioxidant balance ². Increased MDA may be increased risk of



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developing several diseases such as mutations, cancer, inflammation, cardiovascular disorders^{3,4}. In both mammals and fish, insufficient ingestion of nutritional antioxidants can lead to a decrease in the ability to defend against oxidative stress and some diseases⁵⁻⁷.

Paraoxanase (PON) (E.C 3.1.8.1) is a calcium-dependent enzyme catalyzing the hydrolysis of organophosphate compounds and synthesized in the liver^{8,9}. It is associated with high-density lipoprotein (HDL) that prevents oxidative modification of low density lipoprotein (LDL) and HDL. So, PON is responsible for the antioxidant activity of HDL¹⁰. PON is protective against oxidative stress and it reduces oxidative stress in tissues and cells².

HDL is a lipoprotein consisting of 50% protein, 30% phospholipid and 20% cholesterol. HDL molecules are synthesized in the liver and small bowel wall. The newly synthesized HDL is released into the bloodstream collects cholesterol esters from other circulating lipoproteins and the cholesterol esters convert to spherical HDL mature. HDL enriched from cholesterol leaves cholesterol after turning to liver. HDL carries cholesterol from tissue to liver. Therefore, the increase of HDL is in favor of the organism whereas the decrease in HDL levels is against the organism. In human, it was suggested that decreased PON activity in HDL may cause increased oxidative stress indicated by the increase in MDA².

Rainbow trout cultivated in Turkey and in the world is one of the economically important fish species. There is no available information whether there is any relationship between serum HDL, PON and MDA of NRT and ART. This is first study that evaluated levels of MDA and HDL and activity of PON in fish (NRT and ART) living in same pool.

MATERIAL and METHODS

Characteristics of the Water Environment of the Fish Lived

Characteristics of the water environment of the fish lived is an artesian water that has a flow rate of 1 L per in second and has a temperature of 10.5°C, contains dissolved oxygen of 10.6mg/L, has a pH value of 7.4 to 7.8.

Fish, Blood and Analysis

ART (albino rainbow trout) and NRT (normal rainbow trout) reared in the same conditions were obtained from the Fisheries Faculty at Atatürk University in Erzurum. The average weights of fish were 130±10.7g for AT group and 150±11.9g for RT group. Fish were reared in the same pool during four month. Fish sample were randomly caught from pool, and five fish (three replications) were used for each group. ART and NRT were fed with commercial trout feed (crude protein 40-50%, fat 18%, ash 10%, Gross energy 3548 kkal) twice a day.

Blood samples were collected with a syringe from the caudal vein of fish¹¹⁻¹⁵. Clotted blood samples were centrifuged at 3.000 rpm for 10 min. Serum samples are separated and storage at -80°C until analysis. Serum HDL concentration was measured using spectrophotometric method by autoanalyzer Cobas C501 (Roche Diagnostics, Mannheim, Germany) with commercially available kit.

MDA levels were measured according to method described by Aksoy et al.¹⁶. Briefly, A mixture of 20% acetic acid, 0.9% thiobarbituric acid and 8.1% sodium dodecyl sulfate has been added to 0.2 mL of serum, then distilled water has been added to the mixture to bring the total volume up to 4 mL. This mixture was incubated at 95°C for 1 h. Following incubation, the tubes were left to cool under cold water and 1ml of distilled water plus 5 mL of n-butanol/pyridine (15:1, v/v) were added, followed by mixing. The samples have been centrifuged at 4.000 rpm for 10 min. The supernatants were removed, and absorbance was measured with respect to a blank at 532 nm. Malondialdehyde level were expressed as µmol/L.

PON levels were measured according to method described by Börekci et al.¹⁷. Briefly, 40 µL of serum (diluted 1:2 with water) was incubated with reaction mixture at 37°C for 5 min, and then the enzymatic reaction was started by the addition of a substrate solution. The basal assay mixture contained 1.2 mM paraoxon, 1 mM CaCl₂ and 50 mM Tris-HCl (pH 8.0). P-nitrophenol absorbance increase is recorded at 412 nm, ambient temperature 25°C and intervals of 30 sec. The amount of p-nitrophenol formed per minute was calculated from the molar absorption coefficient at pH 8.0 (17600 M⁻¹.cm⁻¹). A quantity of 1U/mL of PON was defined as 1 nmol of p-nitrophenol formed per minute.

Statistical Analysis

To evaluate statistically significant differences between research groups, t-test was used. Statistical analyses were performed by SPSS package program (ver. 11.5). For the comparison of data, P<0.05 was considered statistically significant.

RESULTS

The results that obtained from the serum HDL and MDA levels and PON activities and the results of statistical analyses is given in [Table 1](#) for ART and NRT. Mean PON activity and HDL level in NRT group was higher than in ART group but they were statistically insignificant (P = 0.09 and P = 0.07 respectively). Mean MDA levels were lower in NRT group than in ART ones. The degree of this statistical significance was borderline (P = 0.06).

DISCUSSION

MDA is affected by many factors such as stress, source

Table 1. Levels of MDA and HDL, and PON activity in NRT and ART groups
Table 1. NRT ve ART gruplarında MDA, HDL ve PON aktivite seviyeleri

Parameter	ART	%	NRT	%
MDA (µmol/L)	49.1±4.74		45.2±5.02	
PON (U/ml)	58.4±6.30	100	69.8±5.17	100
HDL (mg/dl)	73.1±4.35	100	86.1±3.0	100
PON/HDL	0.80±0.06	80	0.81±0.05	81

Results were given as mean±SD, MDA: Malondialdehyde; PON: Para-oxanase; HDL: high density lipoprotein; %: rate of PON activity in HDL, and total HDL in the blood and total PON in the blood

in feed, and environmental conditions¹⁶. These factors may lead to weakening of defense system against diseases and oxidative stress. Partial food starvation has been also reported to increase MDA levels in rainbow trout, *Oncorhynchus mykiss*¹⁸, and sea bream, *S. aurata*¹⁹. The levels of MDA, a metabolite derived from lipid peroxidation were examined. Based on MDA levels, our results showed that MDA level of NRT was lower according to ART. There was not any pathological cases or anomalies detected in groups (ART and NRT). However, NRT was a more dominant species in pool according to ART, and the feed intake and growth of ART were slower than the ones of NRT in our study. This may lead to increase of MDA level.

The serum PON activity protects LDL and HDL from oxidation. This protection can relate to the ability of PON to hydrolyze some oxidized phospholipids¹⁰ or cholesteryl linoleate hydroperoxides²⁰. The levels of PON of four neotropical fish species (Dourados, Casudo, Pacu, Matrinxas) were reported as 37.3, 6.1, 6.6, 1.50 nmol/ml²¹. Our results showed that the serum PON level was higher in NRT. In this case, the serum PON may be important in reducing the adverse effects of MDA increased depending on the increase in lipid peroxidation. Follya²² reported that PON activity is related to HDL in serum pacu. In another study, Baston et al.²¹ reported that 95% ration PON/HDL in dourados was recovered in the HDL fraction. In this study, 80-81% rations PON/HDL in ART and NRT were recovered in the HDL fraction (P> 0.05).

HDL is a multifunctional lipoprotein. Besides its obvious contribution in cholesterol transport and metabolism would display an important defensive role against bacterial diseases in carp and trout, and possibly in other teleost fish. In addition, HDL would retain important defensive function, maintaining invariantly high concentrations in the plasma even in diseased fish²³. The mean value of serum HDL for rainbow trout was determined as 114 mg/dL²⁴. The ranges of serum HDL for trouts were determined as 1062-2216 mg/dL for male trout; >1500 mg/dl for 0.7-1.9 kg female trout; 2344mg/dl for immature trout; 518 mg/dL for 0.5-1.0 kg male trout; 331 mg/dL for male trout and 1750 mg/dL for 1.0-1.2 kg female trout²⁵. In the present study, the values of serum HDL in ART were insignificantly lower than in NRT. Feed

intake due to a the dominant species of NRT was higher than one that ART. Therefore, adequate feed intake or size and status of being the dominant of NRT may lead to increase of HDL level.

As a conclusion, The different species and size, dominance status may be reason for the change of PON activity, MDA and HDL levels even though statistically insignificant. The cause of statistically insignificant results may be small number of cases. Further prospective and longitudinal studies with large cases are needed to investigate differences in HDL and MDA of PON in different species of fish living in same pool.

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