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The Effects of Different Stunning Techniques on Blood Biochemistry of Brown Trout (*Salmo trutta fario*)

Muhammed ATAMANALP[1], Murat GENÇ[2], Gonca ALAK[1*], Nilüfer SABUNCUOĞLU[2], Esat Mahmut KOCAMAN[1], Ömer ÇOBAN[2], Asuman ÖZKAN[3], Ziya Gökalp CEYLAN[4]

[1]Department of Aquaculture, Faculty of Fisheries, Atatürk University, TR-25240 Erzurum, Turkey
[2]Department of Zootechny, Faculty of Veterinary, Atatürk University, TR-25240 Erzurum, Turkey
[3]Department of Biochemistry, Regional Education Hospital, TR-25240 Erzurum, Turkey
[4]Department of Food Technology, Faculty of Veterinary, Atatürk University, TR-25240 Erzurum, Turkey

ABSTRACT

In this study, physiological responses of brook trout (*Salmo trutta fario*) were researched after the different stunning methods with biochemical enzymes. Icy water, electroshock, CO_2, hypoxia, and head hitting were tried as stunning techniques to 75 fish and the alterations in the blood biochemistry parameters were analyzed. Alkaline phosphates (*ALP*), and amylase (*AMS*) were found as not statistically important, but alanine transaminases (ALT), aspartate transaminases (*AST*), creatin chitinase (*CK*), *CK-MB* (one of the *CK* isoenzymes), gamma glutamil transferase (*GGT*) and lactate dehydrogenase (*LDH*) levels were important ($p<0.05$). According to these results, the different stunning techniques affected the stress levels and physiological situations of brook trout. The study findings showed that killing techniques resulting in a shorter period were more suitable for animal welfare.

Keywords: Brook trout, biochemistry, *blood*, stunning methods

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*** CORRESPONDING AUTHOR**

galak@atauni.edu.tr
Phone : +90 442 231 2178

Farklı Öldürme Tekniklerinin Kahverengi Alabalık (*Salmo trutta fario*) Kan Biyokimyası Üzerine Etkileri

Öz: Bu çalışmada, kahverengi alabalıklara (*Salmo trutta fario*) farklı öldürme teknikleri uygulanarak balıkların fizyolojik yanıtları bazı kan biyokimya enzimleri ile araştırılmıştır. Buzlu su, elektroşok, CO_2, hipoksi ve kafasına vurma gibi farklı öldürme teknikleri, 75 balık kullanılarak denenmiş ve balıkların kan biyokimya parametrelerindeki değişim gözlemlenmiştir. Bu değişimlerde alkalin fosfataz (*ALP*) ve amilaz (*AMS*) istatistiki olarak önemsiz ($p<0,05$), kreatin kitinaz (*CK*) ve *CK* izoenzimlerinden olan *CK-MB* enzimi $p<0,05$ seviyesinde, alanin aminotransferaz (*ALT*), aspartat aminotransferaz (*AST*), gama glutamil transferaz (*GGT*) ve laktat dehidrogenaz (*LDH*) ise $p<0,05$' de önemli bulunmuştur. Sonuçlar, öldürme tekniklerinin kahverengi alabalıklarda stres kaynağı olabileceğini ve fizyolojik durumlarında etkili olduğunu göstermektedir. Çalışma bulguları, daha kısa sürede sonuçlanan öldürme tekniklerinin hayvan refahı açısından daha uygun olduğunu göstermiştir.

Anahtar kelimeler: Kahverengi alabalık, biyokimya, kan, öldürme teknikleri

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Introduction

Biological and environmental factors may cause stress in aquatic livings. These stress factors may be vital activities as well as various farm practices. Genetic, farm treatments, environmental factors, growth, malnutrition, hunger, stock and transport density, and deformities can be effective in the decreasein fish welfare (Conte 2004; El-khaldi 2010). In response to stress in aquatic organisms, especially fish are widely used in bio-indicator for observing the physical and chemical changes. Because of fish blood results of the stress response in the right behalf of developing secondary effects of stress factors and ecosystem health, a fast and complete results (Kayhan et al. 2009; Atamanalp et al. 2012) Biochemical reactions are catalyzed by enzymes (Kıran et al. 2006). Stress factors provide expression with various metabolic findings such as hormonal, enzymatic and blood parameter alterations. It becomes possible to measure the stress levels in the livings with these parameters. Metabolic activities respond wholly when reached to the level of threshold interaction. The reason for this is that live metabolism works in harmony with the holistic balance.

The metabolic activities of living organisms may vary depending on various reasons in the process of natural life circulation and physical, biochemical, physiological troubles may occur. Stress is the interaction between the factors that make it and the defense reactions of the organism. The stress (internal or external) factors threaten the constant internal balance of the body in animals. The sources of stress are generally factors such as water temperature changes, oxygen deficiency, unsuitable fishing, and handling process. On the other hand, the biological stress factors include stocking density, microorganisms, macro-organisms, sex, other fish species, genetic background and life cycle of fish (Alak et al. 2010a).

The effects of stunning techniques on antioxidant enzyme activities, hematology, and food quality are widely studied (Cui et al. 2012; Botsoglou et al. 2014; Zampacavallo, et al. 2015; Chen et al. 2016; Zhang et al. 2017; Venturini et al. 2018). Because of the limited knowledge about how different stunning techniques affect the blood biochemistry, the stress mechanism and physiology of fish are not fully elucidated (Utrera and Estevez 2012).

The aim of this study is to determine the most suitable stunning technique for brown trout, levels of stress by examining the blood biochemistry parameters and to provide a reference to the studies to be done on this subject of the level.

Materials and Methods

Fish (180 ± 15g weighing 75 brown trout (*S. t. fario*)) were obtained from Atatürk University Fisheries Faculty Inland Water Fish Species Treatment and Research Center, which had no infections and toxic history. Each group designed with 15 fish stockings.

Stunning techniques

Fish were grouped as, one for control others as treatments: Group I: Iced water (2/3 of total volume) (Urbieta and Gines 2000), Group II: electroshock (Roth et al. 2002), Group III: CO_2 treatment, fish were placed in a saturated water tank with CO_2 and gas flow continued throughout the trial (Roth et al. 2002). Group IV: Hypoxic group, accepted as the control group and hypoxia was applied, Group V: Impact on the fish head (Mishima et al. 2005).

Obtaining the blood samples, biochemical and statistical analyzes

Blood of fish samples were obtained from caudal vena with injection approximately 3 ml. placedon biochemical tubes. After centrifuged 10 minutes at 4000 rpm (Bricknell et al. 1999), were analyzed with autoanalyzer for biochemical parameters (*ALT, AST, ALP, LDH, AMS, CK, CK-MB* and *GGT*). The obtained data was applied variant analysis by using the SPSS software (Alak et al. 2010b).

Results

The effects of different stunning methods on the biochemical parameters of brown trout (*S. t. fario)* blood are shown in Tables 1 and 2.

Table 1. Biochemical values and statistical results.

GROUP	*ALP* (U/L)	*AST* (U/L)	*ALT* (U/L)	*LDH* (mg/dl)
Group I	503.83±19.22 a	636.78±10.51 a	13.33±3.85 ab	1455.64±87.65 ab
Group II	438.54±13.45 a	524.50±13.19 ab	9.75±3.25 b	1189.10±47.16 ab
Group III	519.00±14.23 a	545.38±9.33 ab	14.25±9.69 ab	1790.64±26.83 a
Group IV	419.55±13.37 a	481.63±17.03 b	10.25±5.61 b	1403.00±42.91 ab
Group V	465.55±8.18 a	491.13±12.43 b	17.16±6.56 a	1081.63±74.90 b

*****Each value represents mean ±SD (n=15) there is no statistical difference between the values shown in the same column with the same letter (a, b) ($p<0.05$).

Table 2. Biochemical values and statistical results.

GROUP	*AMS* (U/L)	*CK*	*CK-MB*	*GGT* (U/L)
Group I	396.00±21.04 a	5635.92±408.42 a	2256.36±226.45 b	9.91±4.31 a
Group II	397.81±26.99 a	1683.70±144.81 b	2337.00±187.39 b	7.50±3.42 a
Group III	354.00±11.26 a	4998.50±198.56 a	5783.36±196.26 a	10.08±5.68 a
Group IV	255.18±15.53 a	1819.91±115.10 b	2579.11±168.44 b	8.25±4.03 a
Group V	359.90±25.86 a	3964.91±310.91 ab	2492.64±243.92 b	15.25±9.69 b

*****Each value represents mean ±SD (n=15) there is no statistical difference between the values shown in the same column with the same letter (a, b) ($p<0.05$).

The nearest results to the control group (group IV; hypoxic) were obtained in group II (electroshock) and group V (impact on the fish head) in terms of *ALP*, *AST*, *ALT* and *LDH*.

The CK and CK-MB parameters, among the muscle enzymes, were observed the highest value in group I (iced water) and group III (CO_2 treatment). This situation showed that the highest stress conditions were found in these groups.

Discussion

Aquatic livings react to all stress factors in their living environment. These stress factors cause structural and functional disorders in fish blood cells and eritropetic tissues (Witeska and Baka 2002). As a result of the death of the cells, enzymes are released and as a result of sublethal damage, increases can be recorded with the increase of cell membrane permeability and exocytosis of the enzyme (Mert 1996).

Although there was no statistical difference between all groups for the *ALP* value, the control group gave the lowest *ALP* value with 419.55 ± 133.70 (U / L). Apart from other enzymes, the increase in *ALP* activity is induced by stimulation (Mert 1996). Similarly, lowest *AST* value was obtained in control group 481.63±170.30 U/L, the highest *AST* value 636.78±105.09 U/L was in at iced water group and the differences were evaluated significant at the level of $p<0.05$.

Prior studies reported that muscle activities increase *AST* activity (Tekeli et al. 1996). Although there is no result for this value with a similar treatment in the literature scans made, it is thought that the observed increase in all values in the value group is related to severe muscle movements (Mert 1996).

ALT and *LDH* values were obtained statistically important in all groups ($p<0.05$). It is thought that the increase in serum bilirubin levels may be effective in groups in which elevation in *ALT* level is more prominent (Özbek et al. 2006). Findings of the present research, especially *AST* values are similar to the findings which trained different stressors studies (Özbek et al. 2006; Gencer et al. 2015). When the *AST*, *ALT* and *ALP* values are higher in the treatment groups than in the control group, it is thought that the inflammation resulting from stressors in the liver vessels is effective (Özbek et al. 2006). High *CK* levels are effective at the cellular enzymes' increasing as *LDH*. This situation is an indicator of the answers to stress and deformation of muscles (Bórnez et al. 2009). Sabow et al. (2016) reported that high levels of *LDH* are an important indicator of stress-related muscle fatigue and damage.

No data on fish was found in the literature scans for all the values on the chart, so, compared with heavy metal pollution, which is a different stressor. Fırat and Inandı (2016) reported that the concentrations of zinc, cadmium, zinc + cadmium increased the *AST*, *ALT*, *LDH* and *ALP* enzymes of *Oreochromis niloticus*. The values of *ALT*, *LDH*, and *ALP* have increased in the present research, but not statistical importance ($p>0.05$). It has recently been recognized that *AST* and *ALT* are indicative of liver cell damage (Gencer et al. 2015). The main synthesis site is the liver *AST* and *ALT* gluconeogenic enzymes, which are not normally found in serum. Due to adaptation to ambient conditions, the energy requirement is not met from the carbohydrate sources, causing the enzyme synthesis to stop (Fırat and Inandı 2016). Changes in *AST* levels are due to stress factors such as muscular dystrophy, muscle trauma, intramuscular injections, reproductive, hypoxia, stock intensity, and starvation, as well as toxic effects (Hilmy et al. 1985; Vijayan et al. 1997). As a result, whatever the type of stress, the negative effects on vital organs in the body are inevitable. If stress is not removed in time, irreversible damage to the body can occur. A significant increase was obtained in *AST*, *ALT* and *ALP* of the blood samples, obtained from the brown trout, which stunned with different methods in this research. It is known that the stress before death raises the lactate level. By death, catecholamines are released, resulting in the formation of hyperlactitemia as a result of muscle movement and rapid glycogenosis. Increasing the lactase level raises the levels (Svete et al. 2012).

Amylase is known as alpha-amylase (Mert 1996). While the differences among the groups of *AMS* values were evaluated as not statistically important, the highest value (359.90±258.59 U/L) was obtained in a shot on the fish head. The lowest *AMS* value belonged to the control group 255.18±155.28 U/L.

As seen in Table 2, *CK-MB* values of creatine chitinase and creatine chitinase isoenzymes were statistically significant ($p <0.05$) among all groups. The highest values for *CK* and *CK-MB* were belonged to the CO_2 group as in order, 4998.50±1985.60 and 5783.36±1962.58. Literature studies have been carried out in studies conducted with other species in which the *CK* values increase with movement. Pre-cutting applications and cutting techniques are characterized by muscle enzymes - *CK* and *AST* measurements, which are characterized by their effect on the physiological response of the animals (Svete et al. 2012). Changes that involve physical activities before slaughter (jump, etc.) are in behavioral responses to stress conditions and cause muscle damage and muscle enzyme activities to increase with muscle damage (Boissy 1995; Winther et al. 2005; Svete et al. 2012). All groups' *CK* and *AST* values are found higher than control in this research. This suggests that the stunning techniques cause muscle damage and thus increase the serum muscle enzymes. Significant increases in serum *CK* and *AST* activities, however, may also be attributed partly to leakage of the enzyme from the skeleton. Muscle cells are damaged by rapid glycogenolysis induced by catecholamine or increased muscle damage and membrane permeability during slaughter (Svete et al. 2012).

Creatine kinase (*CK*), which has three isoenzymes, is an enzyme found in skeletal, cardiovascular and brain, one of these isoenzymes, *CK-MB* isoenzymium, which is located in the heart and skeleton and constitutes 20% of *CK*. This isoenzyme level raises the myocardia problems. Acute myocardial infarct, myocarditis, cause to increase *CK* level with excessive activity (Vijayan et al. 1997; Werner and Gallo 2008). In our study, 5635.92 ± 4084.22 *CK* value compared to the control for water + ice mixture, which is considered as hypothermia application, was higher than all groups. High *CK* activity is an indication of cell muscle damage and muscle fatigue (Sabow et al. 2016)

GGT is a more sensitive indicator than *ALP* and it is evaluated parallel with ALP and *ALT*. This value raises more and faster than *ALP*. The highest *GGT* score was 15.25 ± 9.69 (U / L) in the group shot on the fish head as it is in the *ALT* group, and the difference between the groups within the value was found to be very important at $p<0.05$. In blood biochemistry, especially bilirubin and *ALP* values are increased. However, there is an increase in *AST*, *ALT*, and *GGT* levels because of liver damage due to cholestasis (Nakyinsige et al. 2013).

Because of the investigation of the present research findings, no significant differences were found statistically between *ALP* and *AMS* values among the control and treatment groups, but significant changes were observed in respect of other blood biochemical parameters (*AST*, *ALT*, *LDH*, *CK*, *CK-MB*, and *GGT*) compared to the treatment and control groups. It is necessary to know the standard parameters of each fish species for use as an indicator of health. In this research, is thought to have contributed to the work to be done to identify the effects of different killing methods on fish welfare. Our results show that short-term killing techniques (Group II and IV) give better biochemical results in terms of *S. t. fario.* welfare and low-stress status.

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