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DNA Damage in Hybrid Tilapia (*Oreochromis niloticus* x *O. aureus*) Exposed to Short-Transport Process

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Abstract

In this study, DNA damage in hybrid tilapia exposed to the short-transport process has been investigated. Gill samples were taken from tilapia which after immediately from the transport process (t0 group), after 6 hours from transport process (t6 group), after 12 hours from transport process (t12 group), after 24 hours from transport process (t24 group) and not applied transport process (control group) have been investigated and the results have been compared as statistically. The Damage frequency (%), Arbitrary Unit and Genetic Damage Index (%) were evaluated in gill cells of tilapia. As a result of the study, it is determined that highest the damage frequencies (%) as 69.00±4.58 and 66.00±3.00 were significantly observed in t0 and t6 groups respectively (P<0.001). Besides, it is observed that the other damage parameters (Arbitrary unit and genetic damage ındex) in the gill samples of t0 and t6 groups were significantly higher (P<0.001) compared to the control, t12 and t24 groups. The Arbitrary unit and Genetic damage index increased in fish after the transport process (especially, t6 and t12) but a significant decrease occurred after 24 h (returning to the control levels) (P< 0.001).

Keywords:
Tilapia, Fish transport, DNA damage, COMET Assay
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Introduction

In fish farming, transfer covers different time procedures. In addition, keeping stress at a minimum for fish businesses is very important for the welfare of the creature. Stress and some stress-related factors cause DNA damage in fish. The difficult situations in which aquatic organisms remain due to environmental conditions cause oxidative stress in the living thing. This increases the risk of fish getting sick and bacterial infections (Sanz et al., 2012). If the change in oxidative balance is not

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adequately repaired by the antioxidant system, the cellular damage that will occur will leave the organism vulnerable to pathological conditions; reproduction, yield and welfare will decrease. It will cause changes in the DNA of the living thing and cause defects in the DNA material. (Sa´nchez-Muros, 2013). Difficulties experienced during transfer also cause serious deaths in fish (Sampaio & Freire, 2016). Fish handling equipment can be variable. Some of these are related to the type of container, density of fish in the container, fish species and transportation time. These features vary according to the type and size of the fish and the distance it will travel. Fish can be transported in plastic bags or large tanks or barrels. Stock density is an important factor during transport because higher densities correspond to lower costs. However, if the increase in density causes death or a high degree of stress that can endanger fish health, it turns out that high density is not economically viable (Reglero et al., 2013; Sampaio & Freire, 2016).

One of the other important factors in breeding establishments has a significant impact on animal welfare (Giovagnoli et al., 2002). Because, exposure of fish to manual handling during transportation, placement in transport environments, deterioration of water temperature, dissolved oxygen and water quality, and adaptation to new places will cause stress in fish. During fish transport, an increase in the ammonia concentration of the transport water may cause acute toxic effects depending on the process (Wicks & Randall, 2002; Akdemir, 2019). Ammonia, from the metabolic wastes of fish, is removed from the body with the help of gills. Ammonia nitrogen can be found in the transport water as non-ionized ammonia (NH3-N) and ammonium ion (NH4-N). Depending on the acidity and temperature of the water, whether or not ammonia is ionized varies. As the acidity and temperature of the transport water increase, the ionization rate of ammonia decreases. While ammonium ion (NH4+-N) cannot pass through the cell wall of aquatic organisms, non-ionized ammonia (NH3-N) can pass through the cell wall. This is defined as the main reason for the toxic effect of non-ionized ammonia in most aquatic organisms (Genç, 2017; Akdemir, 2019).

Due to metabolic activity in fish transfers, the amount of nitrogenous compounds in the transport water is toxic to fish. In this context, the amount of non-ionized ammonia (NH_3) should be less than 0.2 mg/l under cultivation and transportation conditions. When this value is exceeded, toxication occurs in fish (Boyd & Tucker, 1988).

Adaptation problems that occur during transportation in fish cause serious problems that are either reversible or irreversible in metabolism. By limiting many vital functions of fish such as growth, reproduction, development and respiration, it can lead to death by disrupting the homeostatic balance (Borreto et al., 2006; Keleştemur & Özdemir, 2008). In this study, DNA damage in hybrid tilapia exposed to the short-transport process has been investigated.

Materials and Method

The experiment was carried out with hybrid tilapia (average weight of 1.50 ±0.15 g) in the Iskenderun Technical University, Faculty of Marine Sciences and Technology, Aquaculture Research and Development Center, Turkey. One hundred tilapia have been transported from Cukurova University, Aquaculture Research and Development Center to the Iskenderun Technical University, Faculty of Marine Sciences and Technology, Aquaculture Research and Development Center.

Tilapia were caught from the culture tank and held in a depuration tank for 16 h to allow gastrointestinal emptying. Fish weight was not significantly different among treatments. They were then placed in 30-L polyethylene bags with 1/4 water/pure oxygen ratio during fish transport. After transportation, fish were transferred to 500-L tanks provided with continuous aeration and a water recirculation system for the recovery. Fish from each bag was kept in separate tanks for subsequent monitoring. Gill samples were taken from tilapia which after immediately from transport process (t0 group), after 6 hours from transport process (t6 group), after 12 hours from transport process (t12 group) and 24 hours from the transport process not applied transport process (control group) have been investigated.

Comet assay was done according to cellular dissociation technique improved from Cavalcante et al. (2008). Firstly, gill tissues of tilapia were homogenized and centrifuged at 3000 rpm at 4 °C for 5 min for the cell suspension, and then the cell pellet was retained. Singh et al. (1988) was followed for performing the single-cell gel electrophoresis. The slides were neutralized with ice-cold 0.4 M Tris buffer (pH 7.5), stained with 80 ml ethidium bromide (20 mg mL -1). The slides were then examined at X400 magnification using a fluorescence microscope Image2M Zeiss). Images of 100 cells from each sample (gill cell) were visually scored as proposed by Collins, 2004 by classifying the nucleoids. For comparison of the data from the comet assay, the damage percentage (%DF), the arbitrary unit values (AU), and genetic damage ındex (GDI) were calculated as defined by Collins (2004).

For comparison of the data from the comet assay, the damage frequency (%DF), the arbitrary unit values (AU), and genetic damage ındex (GDI) were calculated as defined by Collins (2004). Before statistical treatment, all collected data were tested for normality (Shapiro–Wilk test) and homogeneity (Levene analyze test). Furthermore, a one-way analysis of variance (ANOVA) was applied for significance assessments ($P<0.05$) (Norusis, 1993).

Results

Means and standard deviations of the damage frequency (DF %), arbitrary units values (AU) and genetic damage index (GDI %) of DNA damage in hybrid tilapia from control and short-transport process groups are given in Table 1.

Table 1. Means (%) and standard deviations of DNA damage in the gill cell of hybrid tilapia were obtained from the control and transport process.

Groups	Damage Frequency (%)	Arbitrary Unit (AU)	Genetic Damage Index (%)
Control	29.00±1.00[a]	56.00±2.00[a]	0.56±0.02[a]
t0	69.00±4.58[d]	163.33±18.01[c]	1.63±0.18[c]
t6	66.00±3.00[d]	158.00±13.52[c]	1.58±0.13[c]
t12	42.00±2.64[c]	93.67±9.51[b]	0.93±0.09[b]
t 24	35.333±0.57[b]	67.33±6.65[a]	0.67±0.06[a]
P	***	***	***

The data are shown as arithmetic mean ± standard deviation. *Values with different superscripts in each column indicate significant differences. Indicate significance level between gill tissues of tilapia obtained from control and transport process. (*, P<0.05; **, P<0.01; ***, P<0.001).

No fish mortality was observed at transport process groups and the control during the experiment. In the gill cells of the fish, DNA damage (the damage frequency (%DF), arbitrary unit values (AU) and genetic damage ındex (GDI)) were detected at treatment groups. Significant differences were observed (P< 0.001) in the damage frequency and other parameters (AU and GDI) compared with the control group and transport process groups during the experiment (Table 1).

As a result of the study, it is determined that the highest damage frequencies (%) as 69.00±4.58 and 66.00±3.00 were significantly observed in t0 and t6 groups respectively (P<0.001). The lowest damage frequencies (%) as 29.00±1.00 was significantly obtained in the control group in this study.

Besides, it is observed that other damage parameters (Arbitrary unit and genetic damage ındex) in the gill samples of t0 and t6 groups were significantly higher (P<0.001) compared to the control, t12 and t24 groups (Table 1). The lowest AU and GD were significantly obtained in control and t24 groups in this research. The Arbitrary unit and Genetic damage index increased in fish after transport process (especially, t6 and t12) but a significant decrease occurred after 24 h (returning to the control levels) (P< 0.001).

Discussion

Live fish transport is considered an essential procedure in aquaculture, but often exposes fish to stressors such as air exposure, handling, crowding and confinement (Chandroo et al., 2005; OIE, 2015). Furthermore, it is known to cause the deterioration of transport-water conditions, reducing dissolved oxygen levels and pH and increasing ammonia nitrogen concentrations (Refaey et al.,2017; Refaey & Li, 2018).Fish react to stress by raising the levels of catecholamines and glucocorticoids such as cortisol hormone which is considered a primary stress indicator (Barton & Iwama, 1991). At the cellular level, the formation of reactive oxygen species (ROS) often derives from a stress event (Halliwell & Gutteridge, 1999).These products are biologically generated during metabolism although in stress conditions, their synthesis is greater than the ability of cells to remove them, leading to lipid peroxidation, protein carbonyls' formation, DNA damage and cell

death(Davies, 1995; Winston & DiGiulio, 1991).One of the most important ROS is the superoxide radical, which reacts with nitric oxide giving rise to peroxynitrite, a potent oxidant that may oxidize proteins, lipids and DNA. The revealed rise in the DNA damage in the fish subjected to transportation compared to the parameters in the fish before the experiment suggests that the changes in the gill cells are determined by the cortisol induced damage of the chromosome DNA. Cortisol acts at the genome, inducing somatic mutations in the hemato poietic cells, including erythrocytes, changing their hereditary properties and leading to their malfunctions (Wendelaar, 1997; Kelestemur & Ozdemir 2010, Kamshilova et al., 2013).

Similarly, Turan & Ergenler (2021) examined the effect of the transport process on micronucleus frequency in erythrocytes of carp. They analyzed the blood samples taken from carp immediately after fish transport process (t0 group), 6 hours after fish transport process (t6 group), 12 hours after fish transport process (t12 group) and untransport process (control group) carp and compared the results statistically. The frequency of both micronucleus and nuclear abnormalities can be evaluated in peripheral erythrocytes; As a result of the study, they determined that the highest MN frequency was observed significantly in the t0 group ($p<0.01$). In addition, they found that other nuclear abnormalities (NAs) in blood samples of t0, t6 and t12 groups were significantly higher than the control group ($p<0.01$). Also, Kamshilova *et al.* (2013) studied the effect of transportation on the occurrence of micronucleus in erythrocytes of the peripheral blood of starlet, and reported that the transport process increased the number of fish with micronucleus and the percentage of aberrant erythrocytes.

The study revealed that the increase in the DNA damage parameters in the gill cells indicates the mutagenic effect of transport process on the stability of a genetic apparatus of gill cells. An increased levels of damage frequency, arbitrary unit and genetic damage index in the group of fish subjected to transport process may be explained by the stress hormone-induced lowering of the efficiency of the immune system. Consequently, it has been determined that stress formation in fish can be inevitable during fish transport process and that reversible or irreversible damage can occur by causing various physiological disorders.

Author Contributions

All author contributions are equal for the preparation research in the manuscript.

Conflict of Interest

The authors declare that they have no competing interests

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