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Histopathological Effects of Formaldehyde (CH_2O) on Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792)

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A B S T R A C T

Formaldehyde is commonly used as a disinfectant and also in a control of fish disease in aquaculture sector. However, this widespread use, can lead to environmental degradation and can cause negative effects on the treated fish with. In this study 250 mg/L (1 hour) and 500 mg/L (45 min) concentrations of formaldehyde were used. From the results of the histopathological findings degeneration was determined in the epithelial cells and pilar in the gill lamellae, lymphoid infiltration interlamellar necrosis and degeneration of the muscle tissue, dilatation in the liver, congestion in veins, degeneration in hepatocytes, damage in the blood vessels of fish which were treated with formaldehyde. In conclusion; formaldehyde was found to have a negative impact in histological examination in applied rainbow trout. Therefore, it was concluded that it should be used consciously and according the needs in aquaculture.

Keywords: Rainbow trout, *Oncorhynchus mykiss*, formaldehyde, histopathology

A R T I C L E I N F O

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Gökkuşağı Alabalıklarında (*Oncorhynchus mykiss* Walbaum, 1792) Formaldehit (CH_2O)'in Histopatolojik Etkileri

Öz: Formaldehit su ürünleri sektöründe balık hastalıklarının kontrolü ve dezenfektan olarak yaygın olarak kullanılmaktadır. Ancak, bu durum çeşitli çevresel olumsuzluklara sebep olabildiği gibi, uygulama yapılan balıklarda da olumsuz etkilere neden olabilmektedir. Bu çalışmada 250 mg/L (1 saat) ve 500 mg/L (45 dk) konsantrasyonlarında formaldehit uygulanmıştır. Çalışma sonucunda elde edilen histopatolojik bulgularda formaldehit uygulanan balıkların solungaç lamellalarda pilar ve epitel hücrelerinde dejenerasyon, interlamella alanlarda lenfoid ve mononükleer hücre infiltrasyonu ve nekroz, kas dokularında dejenerasyon, karaciğerlerinde genleşme, venalarında konjesyon, damarlarda hasar ve hepatositlerde dejenerasyon belirlenmiştir. Sonuç olarak formaldehit uygulamasının gökkuşağı alabalıklarında histolojik olarak olumsuz etkilere neden olduğu görülmüştür. Bu nedenle su ürünleri işletmelerinde bilinçli ve ihtiyaç dahilinde kullanılması gerektiği kanaatine varılmıştır.

Anahtar kelimeler: Gökkuşağı alabalığı, *Oncorhynchus mykiss*, formaldehit, histopatoloji

Introduction

In our world, population and consequently rapid increasing of nutrition problems, increasing the importance of fisheries continuously that is a rich protein source. In this context, as well as maintaining the sustainability of the stock with protection of fisheries, a significant portion of the protein needs are taken to be met from aquaculture with increasing production of fisheries obtained by breeding (Birici et al. 2014).

In our country, fish disease has increased in parallel with the development of aquaculture. In plants, taken hygienic and prophylactic measures against these diseases has gained of increasing importance. Using antibiotics and chemotherapeutic agents as a result of too much use of chemicals leaving the undesired level of residues in fish meat and in the environment weakens the defense of fish against pathogens by suppressing the immune system (Grondel et al. 1985; Lundén et al. 2002; Lundén and Bylund 2002).

One of the main objectives of fish farming industry which made intensive cultural practices is made healthy fish production in a way that will not harm the environment and human health and contributes made available for consumption. For this purpose a large amount of disinfectant and chemotherapeutic agents are widely used in the

treatment of microbial and parasites infections and disinfection of tools and equipment in rainbow trout which an important farmed fish. However this widespread use causes a negative impact on environmental degradation as well as on fish made applications. This constitute a major problem in aquaculture (Björklund et al. 1991; Aoki 1992).

Formaldehyde is a colorless and pungent and very good water-soluble aldehyde with chemical formula of CH_2O. Formaldehyde which revealed that the carcinogenic character indicate harmful effects on many systems such as the nervous system, respiratory system and digestive system. Professional groups in industrial field where production of formaldehyde is carry out and used and people working in medical laboratories are confronted with the negative effects of these chemicals (Miyaka et al. 1998).

In aquaculture industry, formaldehyde is beneficial in the treatment of external parasitic infections of the gills and skin and used especially in the treatment of Costia, many other protozoans and monogenetic trematodes (Timur and Timur 2003). However, these chemicals affect the gills of fish and cause physiological disorders as well as toxic effects on internal organs (Uslu and Türkman 1987; Miyaka et al. 1998; Doyuk and Çolakoğlu 2004). Formaldehyde showing adverse effects on reproductive function also has been reported to cause damage to the germ cells (Chowdhury et al. 1992). In an experimental study, it has been shown that formaldehyde exposure disrupts the histological structure of the testis and reduce blood testosterone levels and sperm count (Nordøy 1991; Sarsılmaz et al. 1999).

The aim of this study was investigate the histopathological effects of formaldehyde application in fish that used in rainbow trout (*Oncorhynchus mykiss*) in the treatment of infestations, infection and used as a disinfectant.

Materials and Methods

The experiment was held in fish production facility of Faculty of Fisheries in Süleyman Demirel University in 2009. Rainbow trout used in the study obtained from the Aquaculture unit of Faculty of Fisheries and about 100 fish approximately 180 g weight were used. During the experiment the fish were fed with commercial pellets and 2% of body weight. Both in research experiment, over a total of 12 circular fiberglass tanks were used with 0.6 m^3 capacity and with a volume of 400 L water.

In the study; water with flow rate of 12 L/min, the average temperature of 12 °C, 7.2 of pH and 7.4 mg/L dissolved oxygen were used. After creating treatment groups fish were placed in the tank, the necessary examinations were made after 15-day adaptation period. In this research 250 mg/L (1 hour) and 500 mg/L (45 min) of formaldehyde concentrations were used (Arda et al. 2002; Timur and Timur 2003).

For histopathological analysis of fish, gills and liver tissues were fixed in 10% formalin solution without delay. Prepared paraffin blocks according to the preparation techniques for routine pathological examination preparation, cut with adjusted 5 µm microtome and sections staining with hematoxylin-eosin (*H&E*) were examined by light microscopy (Demir 2001; McMillan 2007). Results are evaluated and important stages are illustrated at microscope with magnification of x200 and x400 times (Luna 1968).

Results

In this group of rainbow trout which applicated with 250 mg/L formaldehyde; damage was observed in the blood vessels in the liver, necrotic degeneration and destruction in the muscle tissue, necrosis in the gill lamellae epithelial cells. The damage and interactions in the gill, liver and muscle tissue of rainbow trout which were treated with 250 mg/L formaldehyde are shown in Figure 1, Figure 2 and Figure 3.

Rainbow trout which applicated with 500 mg/L formaldehyde; damage was observed in the blood vessels in the liver and hemorrhage, necrotic degeneration and destruction in the muscle tissue, necrosis in the gill lamellae epithelial cells. The damage and interactions in the gill, liver and muscle tissue of rainbow trout which were treated with 500 mg/L formaldehyde are shown in Figure 4, Figure 5 and Figure 6.

Discussion

There are many diseases and disorders both put damage the plants in a way of economic loses and in a variety of causes affect negatively the fish results die and disease as in human and veterinarian. In addition, disease factors of fish transmitted tohuman and causes infections and toxicosis. Therefore, fish diseases are important in terms of human health (Arda et al. 2002; Timur and Timur 2003).

In fish farming, appropriate and effective disinfectant are used for prevent occurence and spread of diseases and to destroy the agent causing the infection. In aquaculture formaldehyde is one of widely used chemical in combat and control of diseases. Formaldehyde is generally used as a disinfectant and anti-parasitic agents in the treatment of bacterial infections and fungal disease control (Meinelt et al. 2004; Bayram 2008).

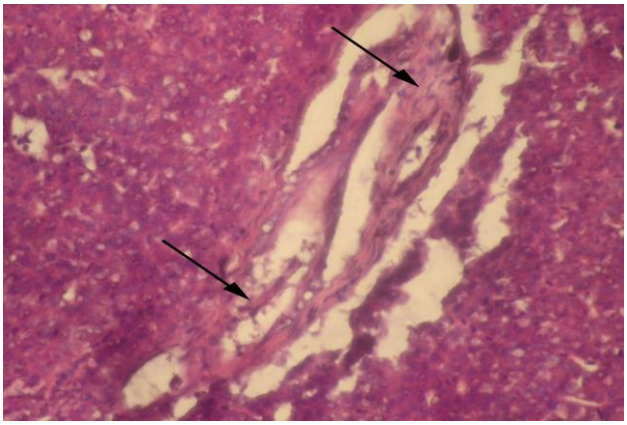

Figure 1. Damage in the blood vessels in the liver of rainbow trout which were applicated with 250 mg/L formaldehyde (*H&E*) (X200).

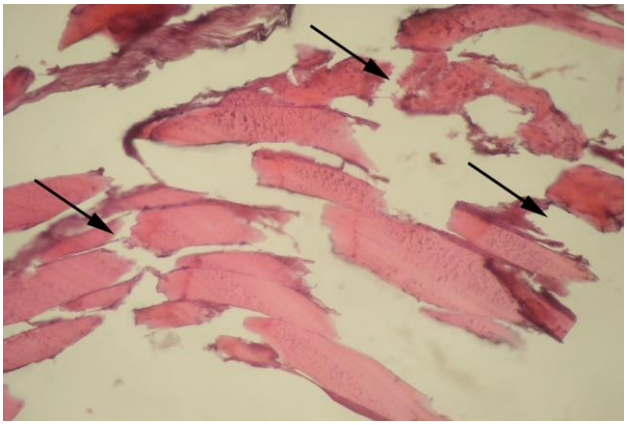

Figure 2. Necrotic degeneration and destruction in the muscle tissue of rainbow trout which were applicated with 250 mg/L formaldehyde (*H&E*) (X200).

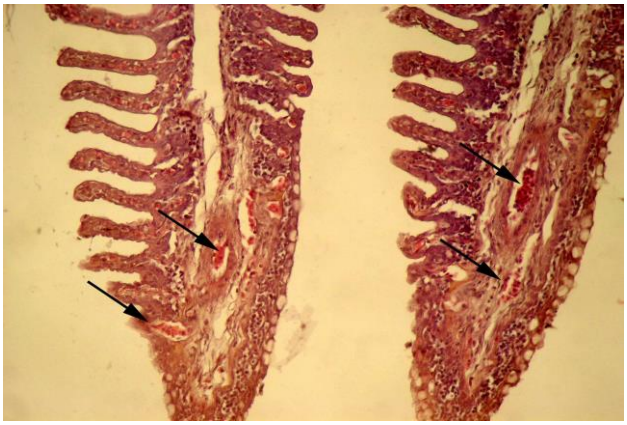

Figure 3. Necrosis in the gill lamellae epithelial cells and hemorrhage in rainbow trout which applicated with 250 mg/L formaldehyde (*H&E*) (X200).

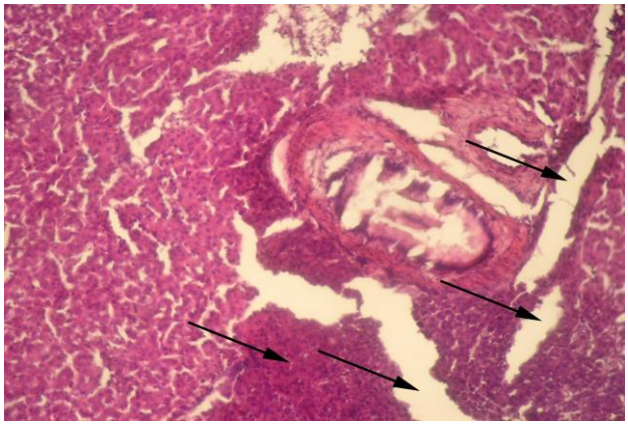

Figure 4. Increased dilatation, hemorrhage and damage was observed in the blood vessels in the liver of rainbow trout which were applicated with 500 mg/L formaldehyde (*H&E*) (X400).

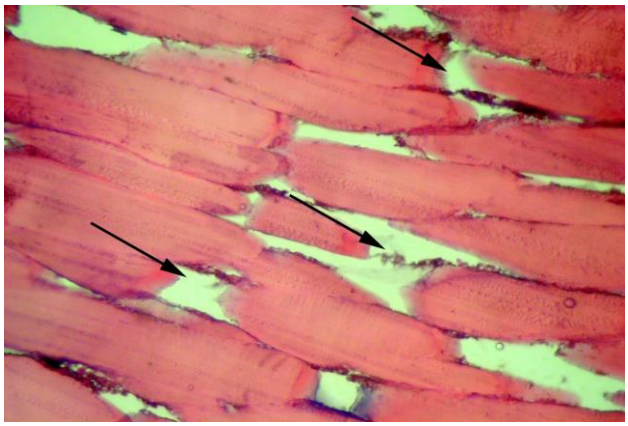

Figure 5. Necrotic degeneration and destruction in the muscle tissue of rainbow trout which were applicated with 500 mg/L formaldehyde (*H&E*) (X400).

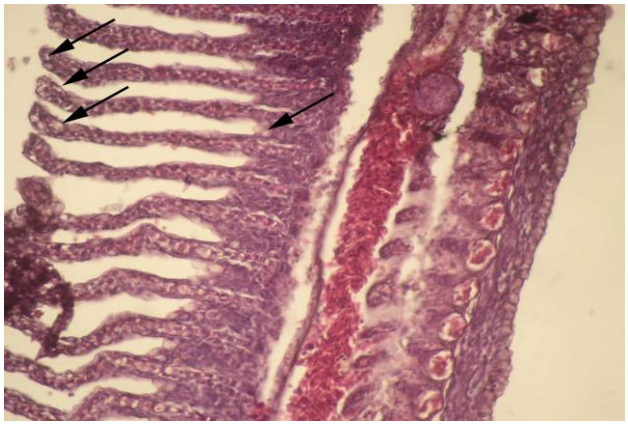

Figure 6. Necrosis in the gill lamellae epithelial cells of rainbow trout which were applicated with 500 mg/L formaldehyde (*H&E*) (X400).

Wedemeyer (1971) was treated the rainbow trout (*O. mykiss*) and coho salmon (*O. kisutch*) with formaldehyde and reported the respiratory as a rate of 10-15%. Wedemeyer and Yasutake (1974) was reported that formaldehyde decreased the dissolved oxygen and cause excessive respiration in fish which exposed to formaldehyde. Williams and Wootten (1981) was reported that 72 h application of 200 mg/L formalin to rainbow trout cause cytoplasmic degradation in liver. Lom and Dykova (1992) was reported that immersion bath which used with formaldehyde in a concentration of 150 mg/L above at 15 °C, 200 mg/L between at 10-15 °C, 250 mg/L at 10 °C and 1hour at 250 mg/L, 24h in flash bath, 15-25 mg/L in constantly baths to be effective.

Cruz and Pitogo (1989) were applied to different formaldehyde concentration ranging between 50 to 500 ppm to the fry (with 6 g average weight) of *Chanos chanos* with the method of static test. The median lethal concentration values (LC_{50}) at 24, 48, 72 and 96 h measured as 322, 260, 241, and 232 ppm respectively. Significant pathological changes were detected in lethal concentrations of the histological analysis of tissue, gills, liver and kidney and it was reported that cell damage violence increases with chemical concentration and exposure. They were reported that hyperplasia, epithelial disruption and necrosis cloudy swelling, hemorrhage and the accumulation of pigments in gill; necrosis in the liver parenchyma and renal tubules and degeneration as histological effect.

Many researchers was reported that the chemicals which used as prophylactic and therapeutic purposes cause stress in cultured fish (Wedemeyer et al. 1990; Barton and Iwama 1991; Pickering 1993; Dursun 1997; Öğüt 2005; Yıldız 2006).

The genotoxic and carcinogenic potential of formaldehyde has been documented in in vivo studies on mammalian cells and rodents. It has been shown that formaldehyde exposure is associated with elevated risks for cancers at various organs and systems, including nasal cavities, lung, and hematopoietic system. It is classified as carcinogenic to humans (Group I) by the International Agency for Research on Cancer (Aydın and Başaran 2012).

Katalay et al (2007) have been reported that more thinning, disruption and degeneration in the lamellae of gill tissue of (*Gobius niger*) living in İzmir bay at a small and large magnifications.

Üçüncü et al. (2010) have been reported that the most significant changes in the liver as steatosis, some fibrous construction and expanding necrotic areas in fish which were exposed to dioctyl adipate (*DOA*) as well as dilatation, poikilocytosis and hemorrhage in central ven. The data presented in that study has shown that the *DOA* is hepatotoxic at least for *Labidochromis caeruleus*.

Our study and the other studies have indicated that the chemicals effective especially on gill and liver.

Sağlam and Ural (2003) have been found findings similar to our study in histological examination in which they treated the rainbow trout (*Oncorhynchus mykiss*) with different concentration of copper sulfate ($CuSO_4$). They examined the rainbow in a point of microscopic and microscopic after subjecting to it 1 ppm, 8 ppm, 16 ppm and 32 ppm concentration. As a result in microscopic findings they have been determined pilar in gill lamellae, degeneration in epithelial cell, vacuolar degeneration and vascular hyperemia and hemorrhage.

It have been concluded that the chemicals have high influence cause similar effect on gill, liver and muscle tissue so the long term use of these chemical can be dangerous, therefore it should be used carefully.

The stress effect that occur in fish may lead to change in physiology, hematology and histopathology of fish (Bulut et al. 2012). Formaldehyde which are used in aquaculture as treatment and disinfectant should be used very carefully. In trout farms, when formaldehyde is used, the used solution shouldn't be poured into the water again. Otherwise, both accumulate as formaldehyde in the tissues and organs of fish in natural environment and the other aquatic organisms and as well as cause pathologic degradation such as detected in this study.

As a result, formaldehyde which is used widely in the treatment of fish diseases may cause respiratory problems during use. Therefore, should be careful when using. During formaldehyde bath applying, it must be attention to the application dose in particular considering the effect on skin and respiratory system of fish.

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