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The Effects of Cadmium on Fatty Acid Composition in the Muscle and Skin of Juvenile Rainbow Trout (*Oncorhynchus mykiss*, Walbaum 1792)

[1]Vahit Konar, [1]Ciler Aydogmus, [2]Ibrahim Orun and [3]Sevket Kandemir
[1]Department of Biology, Firat University, Elazig, 23119 Turkey
[2]Department of Biology, Aksaray University, Aksaray, 68100 Turkey
[3]Science Teaching Programme, Faculty of Education, Amasya University, Amasya, Turkey

Abstract: In this study, the effect of 1 mg L^{-1} (ppm) dose of Cadmium (Cd) on fatty acid composition in the muscle and skin tissues of farmed rainbow trout has been investigated. The effect of Cd was evaluated for 72 h of exposure times. Palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidonic acid (20:4), eicosapentaenoic acid (20:5), docosapentaenoic acid (22:5) and docosahekzaenoic acid (22:6) in fatty acid composition of both skin and muscle tissues have been found. While they were observed that the amounts of palmitic acid, stearic acid increased, palmitoleic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid and docosahekzaenoic acid decreased in the skin and muscle tissues in fish exposed to the Cd compared with control groups. It was also found that Cd had reduced effects on palmitoleic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid and docosahekzaenoic acid belong to unsaturated fatty acid in both skin and muscle tissues.

Key words: *Onchorhynchus mykiss*, fatty acid, cadmium chloride, muscle and skin tissues, enzymes

INTRODUCTION

Cadmium is one of the most important environmental pollutants as a consequence of immense usage in various industrial practices (ATSDR, 1999). It is involved in many industrial uses such as in electroplating, paints, dyestuffs, metallurgical and mining industry and it is now a major danger to man's environment. Cadmium exposure occurs mainly through two sources in the most human population. The first is the oral route through water and food contaminated with cadmium, particularly leafy vegetables, grains, cereals, fruits, organ meat and fish. The second source is through inhalation of cadmium particles during industrial or everyday activities, among which the inhaled Cd^{2+} from cigarette smoke should be considered as highly hazardous because cadmium is easily absorbed by the lungs (Goyer, 1997; Saldivar *et al*., 1991; Stohs *et al*., 1997).

In humans, the efficiency of gastrointestinal absorption of cadmium has been reported to be approximately 3-8% of the ingested load. Cadmium is particularly accumulated in kidney in muscles the concentrations are low (ATSDR, 2003). The endogenous metal binding protein Metallothionein (MT) is known to play a special role in Cd nephrotoxicity by providing Cd a vehicle for transport to kidney and aiding in cellular uptake. The circulating Cd-MTcomplexes, released during liver damage or formed by binding plasma Cd are freely filtered through the renal glomeruli and efficiently taken up by the tubular epithelial cells, where they are rapidly degraded by lysosomal enzymes. The liberated Cd binds to endogenous MT but excess Cd interacts with intracellular machinery to elicit toxicity (Dudley *et al*., 1985; Chan *et al*., 1993; Zalups and Ahmad, 2003).

The existence and induction of MT in the Cd exposured fish tissues including the liver and kidney have been commonly found (Chowdhury and Wood, 2007). The kidney and gills play a vital role in ion-regulation, acid base regulation and nitrogenous waste excretion in fish (Wood, 1993, 1995). It is the primary organ for elimination of water and particularly important for freshwater fish in which efficient ion reabsorption mechanisms in the kidney minimize the accompanying loss of ions (Hickman and Trump, 1969; Larsen and Perkins, 2001). Chronic exposures to waterborne or dietary Cd have been shown to manifest ionoregulatory and other types of physiological disturbances in fish (Chowdhury and Wood, 2007). Cd may act directly on isolated steroidogenic cells responsible for cortisol secretion in rainbow trout (*Oncorhynchus mykiss*) and yellow perch,

Corresponding Author: Sevket Kandemir, Science Teaching Programme, Faculty of Education, Amasya University, Amasya, Turkey

(*Perca flavescens*) (Lacroix and Hontela, 2004). Rainbow trout is one of the most sensitive fish species to environmental pollutants and particularly to Cd (Hansen and Rotella, 2002). Cd ions are taken up through calcium channels of the plasma membrane of various cell types and are accumulated intracellularly due to their binding to cytoplasm and nuclear substances (Saderholm *et al*., 2000; Beyersmann and Hechtenberg, 1997).

It is known that lipids are the main component of cell membranes; therefore alterations in this complex structure could modify the permeability of the cell and generate inadequate nutrients entrance to the cell. One of the mechanism of cadmium toxicity could be through the alteration of lipid synthesis (Alvarez *et al*., 2006). There is considerable information about the effect of Cd on the amount of lipids in different organs (Kumar *et al*., 1996). The Cd treatment resulted in an increase in the proportion of palmitic acid (16:0) and a decrease in linoleic (18:2 (n-6)) acid without changes in the proportion of stearic (18:0), oleic (18:1 (n-9)), arachidonic (20:4 (n-6)) and docosahexaenoic (22:6 (n-3)) acids compared with the control rats. The Cd exposure resulted in a higher relative percentage of saturated fatty acids ($p<0.05$) without changes in the polyunsaturated fatty acids and unsaturation index compared with the control group. Preliminary results from their laboratory appear to indicate that presence of Cd in the intestine decreases the absorption of (14°C) linoleic acid (data not shown). (Larregle *et al*., 2008). It is observed that Cd decreases the amount of phospholipids in peritoneal macrophages (Ramirez and Gimenez, 2002). In particular, it has been reported that the novo synthesis of Phosphatidylcholine (PC) is accelerated by long-term Cd administration after 6 and 12 months in the liver (Waku *et al*., 1985). It has been shown that Cd treatment causes a slightly increase in the rate of the conversion of 18:2-20:4, which is catalyzed by Δ-6 desaturase in cultured hepatocytes (Kudo and Waku, 1996). On the other hand, Cd can affect the metabolism of fatty acids in relation to zinc status. Exposure of zinc-deficient rats to Cd causes a reduction in the activity of hepatic Δ-9-desaturase, which converts 18:0-18:1 (Kudo *et al*., 1990).

The nutritional benefits of fish are mainly due to the content of high-quality protein and high content of the two kinds of omega-3 polyunsaturated fatty acids: Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) in different edible species. Omega-3 fatty acids (EPA) have examined to have protective effects in preventing coronary heart disease, reducing arrhythmias and thrombosis, lowering plasma triglyceride levels and reducing blood clotting tendency (Castro-Gonzaleza and Mendez-Armenta, 2008). The molecular explanation for the anti-arrhythmic effects of n-3 FAs are still a matter of opinion and further studies are required to confirm or exclude the different hypothesis formulated. In fact, these EFAs/PUFAs are able to increase the threshold of ventricular fibrillation, increase heart rate variability and reduce ischemic damage. In the most simplistic interpretation, a very high n-6/n-3 ratio is considered detrimental for human health, while a value as much as possibly close to 1 is considered protective against degenerative pathologies (Russo, 2009).

However, the content of toxic heavy metals in fish can counteract the positive effects of the omega-3 fatty acids present in fish and their beneficial effects on heart disease risk (Chan and Egeland, 2004). The aim of this study was to investigate the effects of 1.0 ppm dose Cd-exposure for 72 h on fatty acids composition of juvenile rainbow trout.

MATERIALS AND METHODS

Experimental animals: The experimental fish, rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) was obtained from a commercial fish farm (Sürgü Rainbow Trout Cage Cultivating Farm, Malatya, Turkey). The fish were fed for 15 days in stock pond (8×5×1.5 m) to acclimatize to the environment. After this adaptation period, ten fish were taken into a 250 gallon tank filled with natural spring water. The fish used in this study had an average 80±0.25 g in weight and 17.2±0.11 cm in length. The composition of the water in the ponds is shown in Table 1.

The water quality test carried out in terms of trace elements by means of ICP-OES spectrometer with graphite oven showed that the water used therein did not have any interference. Fish experiments were performed in accordance with the guidelines for fish research from National Institute of Health and were approved by the Committee of Fish Research at Inonu University, Malatya, Turkey.

Diet preparation: The fish were fed once daily with a commercial trout food (Granulated Hatchery Feed, Corey

Table 1: Some parameters of the water used in the experiment

Water criteria	Before treatment	After treatment
Biological oxygen level (mg L^{-1})	7.56±0.51	7.33±0.42
Chemical oxygen level (mg L^{-1})	28.0±0.400	30.1±0.520
Temperature (°C)	10.6±0.200	10.9±0.250
pH	7.74±0.08	7.86±0.06
Hardness (mg $CaCO_3$ L^{-1})	162.4±4.220	159.1±2.750
Suspended solid (mg L^{-1})	30.1±0.800	34.5±1.100
Total organic carbon (mg C L^{-1})	14.22±1.20	19.30±1.40
Total nitrogen (mg N L^{-1})	1.50±0.03	1.68±0.02
Calcium (mg Ca L^{-1})	117.1±2.500	112.7±2.210
Chlorine (mg Cl L^{-1})	28.5±1.900	26.7±1.600

All data points are the average of n = 3±SD

Feed Mills Ltd, (Na)-6 mg g^{-1} of food; (P = 11 mg g^{-1} of food; crude Protein = 55%; crude Fat = 17%; crude Fiber = 2%) at a rate of 2% body mass per day. Measured food (Ca) was 29.6±1.1 mg g^{-1} of food, n = 14 and measured food (Cd) was 1.5±0.1 μg g^{-1} of food, n = 10). Five days prior to the experiment, the fish were randomly transferred to one of the eight identical 250 gallon polyethylene tanks, where waterborne cadmium and dietary calcium exposures were conducted. Water flow into each tank was maintained at 0.7-0.8 L min^{-1}. Organic debris was siphoned daily. Dead fish were removed daily and mortality was recorded.

Preparation of Cd^{+2} solution: One concentration (1.0 ppm) of diluted salt of cadmium chloride ($CdCl_2$. $5H_2O$) were used in the experiment. The specified doses were released from a prepared stock solution into the 250 gallon aquariums by using a pump. Separate control groups were designated for each one of the two metal applications and these control groups have not been exposed to any metals. In both metal applications, the fish were exposed to the respective metals for periods of 72 h. The fish had not been fed 12 h prior to the experiment.

The experiment was implemented in two series as cadmium applications. The sample fish in each of the series were divided into 4 groups and the first groups in each were designated as the control. The experiment was done to determine the effects of cadmium on fatty acid components in the muscle tissue and the skin of rainbow trout that were exposed to one dose for 72 h.

Extraction of total lipid from tissues: The methods explained in Christie (1992) were used for the extraction of total lipid from the fish tissues, for the elimination of impurities which were not lipid and for the preparation of fatty acid. In this research, SHIMADZU GC 17 Ver. 3 gas chromatography 25 m lenght, 0.25 μm ID PERMABOND 25 MACHERY-NAGEL (Germany) capillary colon used. During analysis, column temperature was maintained at 130-220°C and injection temperature and detector temperature were stabilized at 240 and 280°C, respectively.

Statistical analysis: Statistical analysis was carried out by using the SPSS for Windows, Ver. 10 (SPSS Inc. Chicago, IL, USA). The data obtained are expressed as Mean±Standard Deviation (SD). Student's t-test was used to determine whether differences between means were significant, with $p<0.05$ taken as the significant level.

Chemicals: All the chemicals used in the study were analytical grade and were purchased from Merck (Darmstad, Germany).

RESULTS AND DISCUSSION

In this study, both total lipid and fatty acid levels in the muscle and skin were statistically found significant in animals exposed to 1.0 ppm waterborne Cd compared with control animals ($p<0.05$).

It was showed that sublethal dose of Cd decreased total lipids in both tissues of experimental animals (Fig. 1). The saturated fatty acid (16:0 and 18:0) levels increased, but unsaturated fatty acid (16:1, 18:1, 18:2, 18:3, 20:4, 20:5, 22:5 and 22:6) levels decreased in the skin and muscle of animals exposed to Cd compared with control group (Fig. 2 and 3).

One ppm dose of Cd exposure caused the change of the ratio of omega-3/omega-6 in muscle and skin of fish. This rate was 1.9917 in the muscle and 2.6620 in the skin of control fishes but it raised to 2.8516 in the muscle and 2.8994 in the skin of fishes exposed to 1 ppm dose of Cd.

The nutritional benefits of fish are mainly due to the content of high quality protein and high content of the two kinds of omega-3 polyunsaturated fatty acids.

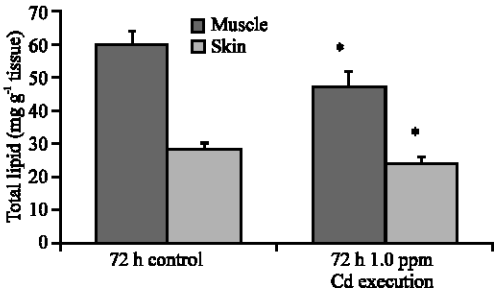

Fig. 1: The comparison of lipid levels in skin and in muscle of animals between control group and 1.0 ppm dose of cadmium for 72 h exposed group (*$p<0.05$)

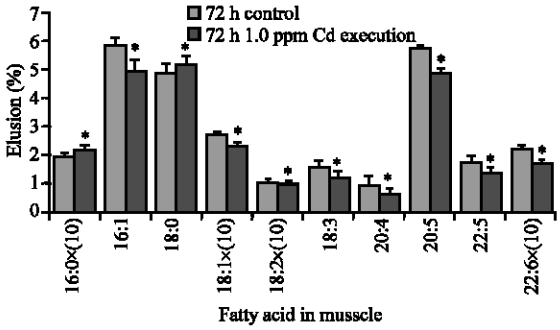

Fig. 2: The comparison of saturated and unsaturated fatty acid levels in skin of animals between control group and 1.0 ppm dose of cadmium for 72 h exposed group (*$p<0.05$; **$p<0.01$)

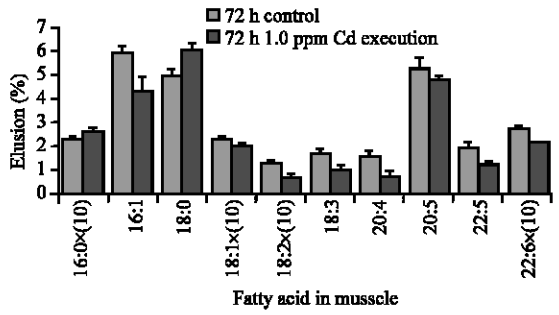

Fig. 3: The comparison of saturated and unsaturated fatty acid levels in muscle of animals between control group and 1.0 ppm dose of cadmium for 72 h exposed group (*p<0.05; **p<0.01)

Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) in different comestible species. Omega-3 fatty acids (EPA) have examined to have protective effects in preventing coronary heart disease reducing arrhythmias and thrombosis. Toxic heavy metals in fish can damage the positive effects of the omega-3 fatty acids present in fish and their beneficial effects on heart disease risk (Chan and Egeland, 2004).

In this study, we found that both total lipid and fatty acid levels in the muscle and skin were changed significant in animals exposed to 1 ppm Cd compared with control animals**.** It was showed that 1 ppm dose of Cd decreased total lipids in both tissues of experimental animals (Fig. 1). The finding showed similarity with the results of Pierron *et al.* (2007), Larregle *et al.* (2008) and Garg *et al.* (2009). The reduction of total lipids in the tissues of animals exposed to Cd may be caused the increase of lipase activity depending on increase of lipogenic enzymes. Cd causes an impairment of lipid storage in the tissues. This impairment appeared to be mainly explained by a Cd effect on lipid mobilization. Pierron *et al.* (2007) and Garg *et al.* (2009) showed that Cd seems to trigger an increased lipolysis at the genetic level as well as the enzymatic level.

The saturated fatty acid (16:0 and 18:0) levels increased but unsaturated fatty acid (16:1, 18:1, 18:2, 18:3, 20:4, 20:5, 22:5 and 22:6) levels decreased in the skin and muscle of animals exposed to Cd compared with control group (Fig. 2 and 3).

One ppm dose of Cd exposure caused the change of the ratio of omega-3/omega-6 in muscle and skin of fish. This rate was 1.9917 in the muscle and 2.6620 in the skin of control fishes but it raised to 2.8516 in the muscle and 2.8994 in the skin of fishes exposed to 1 ppm dose of Cd.

It was noted that lipid peroxides increased in animals exposed to Cd (Kawamoto *et al.*, 2007). Lipid metabolism and fatty acid composition affected depend on peroxidation in animals (Ramirez and Gimenez, 2002). In the present study, it was showed that lipid level decreased in muscle and skin of fish exposed to Cd (Fig. 1). It is known that an increased GPAT, (glycerol-P acyltransferase) activity would lead to an increment in the amount of 1-acyl-sn glycerol-3-phosphate, known to be an early substrate of TG synthesis pathway (Coleman and Lee, 2004).

Lipid peroxidation is the reaction of oxidative deterioration of membrane polyunsaturated fatty acids. The reduction of unsaturated fatty acids in muscle and skin of fish in this study showed similarities that of Kawamoto *et al.* (2007). The total mono and total polyunsaturated fatty acids significantly decreased after cadmium treatment compared to control group. This decrease may be due to cadmium induction of prostaglandin biosynthesis pathway (Choi *et al.*, 2002; Figueired *et al.*, 2002). Cadmium treatment markedly increased the activities of phospholipase A2 and cyclooxygenase 2 (COX-2) enzymes, without affecting COX-1 expression. Also, the activity of 5-lipoxygenase and the synthesis of platelet thromboxane A2 were increased by cadmium treatment (Choi *et al.*, 2002). The metabolic pathways alterations could play a significant role in decreasing polyunsaturated fatty acids levels. Previous study suggested that the triglycerides serve primarily a storage function with toxicity deriving mainly from long-chain Nonesterified Fatty Acids (NEFA) and their products such as ceramides and diacylglycerols (Weinberg, 2006). The total mono and poly-unsaturated fatty acids were significantly decreased after cadmium treatment compared to control group. This decrease might be due to induction of prostaglandin biosynthesis by cadmium. This suggestion was confirmed by Choi *et al.* (2002) and Figueired *et al.* (2002) both of whom found that cadmium treatment markedly increased the activities of phospholipase A2 and cyclooxygenase 2 (COX-2) enzymes without affecting COX-1 expression.

An increase was observed on saturated fatty acid levels in muscle and skin of fish exposed to Cd (Fig. 3). This result showed similarity with the results of Newairy *et al.* (2007) and Larregle *et al.* (2008). The increase in saturated fatty acids content may be due to inhibition of some desaturase enzymes. Supporting this finding, cadmium treatment suppressed activity of hepatic steroyl-CoA desaturase (Alvarez *et al.*, 2006) as well as the activity of microsomal Δ-9 desaturase (Kudo and Waku, 1996). The changes in the distribution of lipids in the different liver subcellular particles after Cd treatment should be associated to a change in the turnover of lipids in a medium high of oxidative stress which is known to modify the properties of membranes (Nigam *et al.*, 1999).

CONCLUSION

In this study, the findings showed that the exposure of heavy metals such as Cd has inhibitor effects on enzymes charged for Δ-9 desaturase and Δ-6 desaturation steps as a result of their reduced effects on unsaturated fatty acid synthesis. As a conclusion, it may be stated that Cd application has effects on the fatty acid components in exposed tissues by preventing the fatty acid desaturation.

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