Harmful effects of waterborne nonylphenol on reproductive performance in rainbow trout *Oncorhynchus mykiss*

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Abstract

In this study, the effects of different concentrations (0, 5, 10, 25, and 40 μg L^{-1}) of nonylphenol (NP) for 90 days on the physiological and reproductive parameters in juveniles of rainbow trout were investigated. In the group exposed to 40 μg L^{-1} of NP, plasma concentrations of 17β-estradiol (E2) and 11- ketotestosterone (11-KT) were significantly lower on days 45 and 90 of the experiment in comparison to the control group. With the increase of NP concentration in the environment, plasma concentrations of cortisol were increased on days 45 and 90 of the experiment. At the end of the experiment, in the groups which were exposed to 25 or 40 μg L^{-1} of NP, gonadosomatic index (GSI) and hepatosomatic index (HSI) were decreased and increased respectively, compared to the control group. The results of the recent study confirm the disruption of the endocrine and metabolic organs in rainbow trout exposed to NP in the early stages of growth.

Keywords: Nonylphenol, Reproduction, Metabolism, Rainbow trout

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Introduction

Nonylphenol is a nonionic surfactant obtained from the biodegeneration of nonylphenol polyethoxylates. The solubility of NP in water is low and it is considered one of the stable pollutants in nature (Ekelund *et al*., 1993). Since NP has a great ability for bioaccumulation, it can have toxic effects on organisms by contaminating aquatic ecosystems even at low concentrations (Ahel *et al*., 1994). If NP enters the human body directly or indirectly through the consumption of contaminated seafood, it can endanger human health. The European Union has set the maximum allowed concentration of NP in water at 2 μg L^{-1} (EU, 2013). It has been shown that NP has effects similar to estrogens and androgens in fish and affects the growth process and reproductive performance (Vasquez-Duhalt *et al*., 2005). Nonylphenol and its derivatives are generally introduced into the environment from the untreated effluents of factories producing household cleaning products, cosmetics, pesticides, plastic, paper, and paints (Lerner *et al*., 2007). Plastic containers left in nature are also a source of NP (Ademollo *et al*., 2018).

Estrogen is one of the most important sex steroids that affects the physiological functions of the body through the hypothalamus-pituitary-gonadal axis (Vetillard and Bailhache, 2006). In male fish, 11-KT as a steroid hormone plays an essential role in the occurrence of secondary sexual characteristics (Devlin and Nagahama, 2002). Vitellogenin (VTG) is a yolk precursor protein that is produced in the liver under the influence of estrogen secreted from the ovaries of the fish. If male and female juvenile fish are exposed to exogenous estrogen sources, they will produce VTG (Maltais and Roy, 2014). Therefore, it is possible to measure the estrogenic potential of NP by measuring VTG in the blood of the fish.

Cortisol is a corticosteroid hormone that regulates the osmotic pressure of body fluids and modulates the response of the body to stress (Mommsen *et al*., 1999). Measurement of cortisol in the blood is used as an indicator of fish response to stress (Wendelaar Bonga, 1997; Bouyoucos *et al*., 2021). Gonadosomatic index and HSI are often used to determine environmental risks affecting fish health (Louiz *et al*., 2009).

The hypothesis of the recent study was that NP induces disturbances in the physiological and reproductive functions of rainbow trout. The aim of the recent study was to investigate the effect of different concentrations of NP (0, 5, 10, 25, and 40 μg L^{-1}) for 90 days on the physiological and reproductive functions of juvenile rainbow trout.

Materials and methods

The research was conducted in accordance with the principles of local Bioethics Committee of Veterinary Faculty of Kermanshah University. One hundred fish with an average age of 60±5 days were randomly placed in $2m^3$ tanks (5 tanks). Freshwater was poured into the tanks and the water temperature was kept constant at around 10°C. The water flow rate was 1.5 lit/min. Fish were fed with a commercial trout diet (Le Gouessant, Lamballe, France) 5 times a day. Fish were reared under natural photoperiod conditions during the experiment. The experiment was conducted from May to July 2021. Nonylphenol with 95% purity (Sigma-Aldrich, Inc. St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, Inc.) to obtain a 0.01% solution. The fish were placed in tanks with different concentrations of NP (0, 5, 10, 25, and 40 μg L^{-1}) for 90 days. On days 45 and 90 of the experiment, 10 fish were removed from each tank and evaluated. Fish blood samples were collected through heart puncture in heparinized tubes (Sarstedt, Numbrecht, Germany). Blood samples were centrifuged and plasma samples were stored at -20°C. Then the liver and gonads were removed from the body to calculate HSI and GSI, respectively from the following equation:

Liver/Gonad weight(g) / total fish weight(g) × 100

Vitellogenin value was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource Inc., USA). Estrogen, 11-KT, and cortisol values in the plasma were also measured by ELISA procedure (all, Cayman Chemical Company, MI, USA). Data analysis was done using a SAS (SAS Institute Inc. 2014. SAS® OnDemand for Academics: User's Guide. Cary, NC: SAS Institute Inc.) statistical software.

Results

The effect of different NP concentrations during the experiment on physiological and reproductive parameters of juvenile rainbow trout is shown in Table 1.

Table 1: The effect of different NP concentrations on physiological and reproductive parameters of juvenile rainbow trout

Parameters (mean±SD)	Exposure day									
	45					90				
	NP concentration (μg L^{-1})									
	0	5	10	25	40	0	5	10	25	40
Plasma E2(pg/mL)	400±11^a	430±22	720±32^b	420±18	330±15^c	440±13^a	460±34	392±34	310±14^b	270±18^c
Plasma 11-KT(pg/mL)	110±7	98±6	143±7	101±5	80±9	97±5^a	120±6	87±4	68±4	50±3^b
Plasma VTG (ng/mL)	1200±32^a	1300±42	2900±33^b	3300±27^c	3900±47^d	1183±31^a	1800±23	3800±37^b	3300±38^c	2900±34^d
Plasma cortisol (ng/mL)	32±8^a	27±6	91±7^a	148±10^b	197±11^c	26±7^a	29±6	83±6^b	75±6^c	68±6^d
GSI(%)	2.4±0.1	2.5±0.1	2.2±0.2	2±0.2	1.9±0.1	3.1±0.2^a	2.6±0.2	2.5±0.2	2.1±0.2^b	2±0.1^c
HIS(%)	1.8±0.1	1.9±0.1	1.7±0.1	1.9±0.2	2±0.2	2.2±0.2^a	2±0.1	2.3±0.2	2.6±0.2	3±0.2^b

Different superscripts in each row indicate statistically significant differences with the control group ($p<0.05$). E2: Estradiol; NP: nonylphenol; 11-KT: 11- ketotestosterone; VTG: Vitellogenin; GSI: gonadosomatic index; HIS: hepatosomatic index. Ten fish were evaluated for each group for each exposure day.

Discussion

The results of the recent study showed significant negative effects of NP on reproductive performance of rainbow trout. The effect of NP on plasma E2 levels was dependent on the concentration of NP and duration of fish exposure in the current study. For the period of 45 days, with the exception of 40 µg L^{-1}group, other concentrations of NP increased plasma E2 levels compared to the control group. The results of a study showed that injection of NP (1-5 mg kg^{-1}, i.p.) significantly reduced plasma concentrations of E2 in Atlantic salmon after two weeks compared to the control group (Arukwe *et al*., 1997). The results of another study showed that exposure to high concentrations (~ 85 µg L^{-1}) of NP significantly reduced plasma concentrations of E2 in female rainbow trout, while exposure to low concentrations (<8 µg L^{-1}) of NP did not have a significant effect on plasma concentration of E2 (Harris *et al*., 2001). The results of a study showed that changes in the plasma concentrations of E2 due to NP were dependent on the age and stage of sexual development of fish (Saravanan *et al*., 2019). In the recent study, it was found that different concentrations of NP have different effects on plasma concentrations of E2. It was demonstrated in the recent study that NP changes the pathway of steroid biosynthesis in fish.

In the recent study, it was found that with the increase of NP concentration, the plasma concentration of 11-KT was decreased. Similar results have been obtained by other researchers in other fish species (Nicholas *et al*., 2001; Zheng *et al*., 2019). The mechanisms of reduction of plasma concentrations of 11-KT due to NP in fish are not well known. Nonylphenol may have a direct toxic effect on the cells producing 11-KT or it may have an indirect effect through the hypothalamus-pituitary-gonads axis.

It was demonstrated in the recent study that with increasing the concentration of NP, the plasma concentration of VTG was increased significantly. Nonylphenol can directly bind to E2 receptors and stimulate the transcription of E2-responsive genes (Kime, 1999). The results of a study showed that there is a relationship between the plasma values of VTG and the expression of the vtg gene in the liver, which is under the influence of E2. Nonylphenol by mimicking the E2 stimulates the production of VTG (Saravanan *et al*., 2019). In the recent study, plasma concentrations of cortisol were increased significantly with increasing the levels of NP. Plasma concentration of cortisol is one of the most important indicators of level of stress in fish exposed to toxic compounds. A High concentration of NP may increase plasma concentration of cortisol by affecting the function of the kidneys. Other studies have also shown the relationship between NP concentrations and plasma concentrations of cortisol in fish (Senthil Kumaran *et al*., 2011; Palermo *et al*., 2012).

It was found in the recent study that long-term (90 d) exposure to high concentrations (25 and 40 μg L^{-1}) of NP significantly reduced the GSI. Other studies have also shown a decrease in the GSI after exposure of fish to NP (Sayed *et al*., 2012; Saravanan *et al*., 2019). The decrease in GSI in fish exposed to NP can be a reflection of decreased activity of the hypothalamus and pituitary glands, or occurrence of morphological/pathological changes in the gonads due to the toxic effects of NP (Sayed *et al*., 2012). The reduction of GSI caused by NP can have negative effects on sexual maturity.

In the recent study, with the increase of NP, HSI was increased too, which can be explained by the stimulation of NP to produce VTG in the liver. It has been demonstrated that the accumulation of VTG in the liver causes liver damage and increases HSI in fish (Barse *et al*., 2006). Excessive production of VTG can reduce the growth rate of fish because a lot of nutrients are spent on the production of VTG (Servos, 1999).

In conclusion, the results of the recent study showed that NP has direct and indirect harmful effects on the endocrine system and body organs of the rainbow trout.

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