Environmental Science and Pollution Research (2022) 29:44513–44522
https://doi.org/10.1007/s11356-022-18995-5

RESEARCH ARTICLE

Effects of long-term exposure of norfloxacin on the HPG and HPT axes in juvenile common carp

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Received: 27 September 2021 / Accepted: 27 January 2022 / Published online: 8 February 2022

Abstract

Currently, there is a relatively lack of relevant research on the interference effect of quinolone antibiotics on the endocrine of aquatic animals. In this study, the toxicity of norfloxacin (NOR) on the endocrine system of juvenile common carp (*Cyprinus carpio*) was evaluated, as well as the hematocyte parameters. Specifically, two important endocrine axes were assessed: the hypothalamus–pituitary–thyroid (HPT) axis and hypothalamus–pituitary–gonadal (HPG) axis. Norfloxacin was used as a representative of quinolone antibiotics. According to the concentration of water pollution areas and considering the bad situation that may be caused by wastewater discharge, a control, 100 ng/L NOR, and 1 mg/L NOR treatment groups were set up. The juvenile carp, as the test animal, was subjected to an exposure experiment for 42 days. Thyroid hormones (T3 and T4) and related genes in HPT axis and sex hormones (11-ketotestosterone [11-KT] and progesterone [PROG]) and related genes in HPG axis and blood count are tested. It was found that the T4 iodine level and conversion process were enhanced after NOR treatment, which in turn led to the increase of T3 content and biological activity in the blood. One hundred nanograms per liter NOR can inhibit the level of sex hormones and inhibit the expression of HPG axis-related genes. In the 1 mg/L NOR treatment group, long-term exposure over a certain concentration range may lead to the development of adaptive mechanisms, making the changes in hormones and related genes insignificant. In conclusion, this study provides reference data for the endocrine interference of quinolone antibiotics on aquatic organisms, and has ecological significance for assessing the health of fish populations of quinolone antibiotics. However, the specific sites and mechanisms of action related to the effects of NOR on the endocrine system remain unclear and require further study.

Keywords Norfloxacin · Juvenile common carp · Endocrine system · Toxicity

Introduction

As an emerging pollutant in the marine environment, antibiotics have caused widespread concern due to their persistence in the environment and their potential risks to aquatic organisms (Prasannamedha and Kumar 2020, Zhang et al. 2020). Quinolone antibiotics are widely used to treat humans, livestock, and poultry because of their broad antibacterial spectrum, limited side effects, and low resistance (Girijan et al. 2020). The concentration of fluoroquinolone drugs in an aqueous environment is usually at the ng level (Xie et al. 2019). For example, the concentration of norfloxacin detected in Laizhou Bay of China was 103 ng/L, and the concentration of enrofloxacin was 209 ng/L (Zhang et al. 2012). However, 0.1010 mg/L of ciprofloxacin was detected in Swedish hospital sewage in heavily polluted areas (Lindberg et al. 2004). Ciprofloxacin is as high as 0.2366 mg/L in Indian waters (Diwan and Tamhankar 2009). However, the discharge of wastewater into the environment in many countries poses a great risk to the ecosystem (Yang et al. 2020).

Norfloxacin (NOR), is a second-generation synthetic fluoroquinolone drug (Chen et al. 2017), widely used in China's aquaculture industry. Its ecological risks are worthy of attention. Studies have shown that NOR can destroy the antioxidant system of phytoplankton, induce oxidative

Responsible Editor: Bruno Nunes

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stress, and affect the growth and behavior of zooplankton (Nie et al. 2009; Pan et al. 2017). To aquatic animals, NOR can induce DNA damage in male goldfish (Liu et al. 2014). NOR has a negative impact on the defense function and intestinal health of juvenile large yellow croaker (Wang et al. 2020). However, there are few reports about the effects of fluoroquinolone antibiotics on the endocrine-reproductive system. Therefore, it is necessary to explore whether quinolone antibiotics, as a typical organic pollutant, can affect the endocrine-reproductive system of fish.

The hypothalamus–pituitary–thyroid (HPT) axis is an important endocrine axis in fish; it is used to regulate the synthesis, secretion, storage, and transportation of thyroid hormones and thereby affects the growth and metabolism of fish (Peter 2011). Thyroid hormones, such as T3 and T4, are important secreted products of the HPT axis in fish (Li and Li 2020, Yu et al. 2020). They play important roles in regulating physiological processes such as growth, development, behavior, and energy metabolism. The synthesis and secretion of thyroid hormones (mainly T3 and T4) are affected by the expression of corticotropin-releasing hormone (*crh*), thyrotropin-releasing hormone (*trh*), thyroglobulin (*tg*), transthyretin (*ttr*), type I deiodinase gene (*dio1*), and type II deiodinase gene (*dio2*) in HPT axis. By contrast, the hypothalamus–pituitary–gonadal (HPG) axis is used to regulate the synthesis, storage, transportation, and metabolism of gonadal hormones (Kanda 2019). In bony fish, sex hormones such as progesterone and testosterone secreted by their gonads, that is, steroid hormones, play an important role in regulating fish gonadal differentiation, gametogenesis, and reproduction (Devlin and Nagahama 2002). Endocrine disruptors can interfere with the synthesis of endogenous hormones through receptor-mediated pathways (Gao and Wang 2014). Genes related to sex hormone synthesis and reproduction such as aromatase (*cyp*19b), follicle-stimulating hormone receptor (*fshr*), synthesis rapid regulator protein (*star*), and vitellogenin (*vtg*) deserve attention (Kanda 2019).

Although quinolone antibiotics are widely used and frequently detected in aquatic environments, the majority of ecotoxicological data on these drugs come from acute toxicity studies. Thus, a large information gap is preventing an accurate assessment of the potential ecotoxicological risks caused by quinolone antibiotics in the water environment. We used carp as a model animal, set up control, 100 ng/L NOR, and 1 mg/L NOR treatment groups, and conducted a 42-day exposure experiment to evaluate the chronic toxicity of norfloxacin (NOR) to its endocrine system at environmental concentrations. We examined levels of thyroid hormones (T3 and T4) and sex hormones (11-ketotestosterone [11-KT] and progesterone [PROG]) and determined gene expression changes related to the endocrine system. The innovation of this research lies in the following points. (1) The concentration setting of the treatment group is more realistic. It is set according to the ng level detected in the water environment and taking into account the bad situation of up to the mg level in the discharged wastewater. (2) There are few reports on the toxicity of NOR to the endocrine-reproductive system. This study provides more theoretical data for future ecological risk assessments.

Materials and methods

Chemicals and test fish

Norfloxacin (98%, CAS: 70458–96-7) from Macklin (Shanghai, China) was used as a toxicant to carp. Juvenile carp (30–40 g) were puchased from Xinda fish farm (Tianjin). We set the water quality parameters (pH 7.6, temperature 23 °C) and carried out temporary breeding for 3 weeks in the laboratory to adapt the carp to the living environment. We made the carp fed with commercial feed (Xinda, Tianjin, China) twice a day, and the waste and residues were removed when changing water every 3 days. The Animal Ethics Committee of Shandong University has approved this study, and it was carried out in strict accordance with the guidelines approved by the Chinese Society of Laboratory Animal Sciences.

Experimental design

The experiment set up control, 100 ng/L NOR, and 1 mg/L NOR treatment groups; each group had three repeated glass tanks, and each glass tank was placed 25 carp. In the experiment, the water quality conditions, feeding, and water change were consistent with the conditions during the holding period. According to the analyzed results, the measured concentration of NOR (93.84 ± 10.52 ng/L and 0.89 ± 0.92 mg/L, corresponding to the 100 ng/L and 1 mg/L) was within 20% of the nominal concentration, which meets the OECD guidelines (the OECD Guideline for testing of chemicals No. 204, "Fish, Prolonged Toxicity Test"). This sampling method is based on the principle of reducing the number of live carp samples and increasing animal welfare. After 42 days, the carp was dissected and the liver, kidney, and gonadal tissues were taken out. The specific sampling method is as follows. Stop eating 24 h before sampling. The carp is anesthetized with MS-222, and blood is taken from the tail of the fish with a syringe and processed into blood smears for red blood cell count, white blood cell count, and white blood cell classification. Take 200μL blood into an anticoagulation tube and mix it thoroughly (whole blood: 1% heparin sodium anticoagulant = 20:1). After standing for 2 h, centrifuge, 2500r/min, 10 min. The supernatant is plasma, which is stored at − 80 °C for hormone detection. During the 42-day sampling, the kidneys, liver, and gonads were quickly taken out on ice and frozen in liquid nitrogen after the blood was taken. After sampling, transfer the tissue sample in liquid nitrogen to − 80 refrigerator for storage.

Hemocytes measurements

The red blood cells and white blood cells were directly counted under the microscope (Learing Resources es-44sm, China) using a hemocytometer. Differential leukocytes were classified and counted using Giemsa (Nanchang Yulu, China) staining. We observe under 1000 times magnification of an ordinary optical microscope.

Thyroid hormone and sex hormone measurement

The plasma samples were used to detect the levels of thyroid hormones(T3 and T4) as well as sex hormones (PROG and 11kT) with the test kits purchased from Chundu (Wuhan Chundu Biotechnology Co., Ltd., China). All measurements were performed using a microplate reader (Tanon, China).

Quantitative real-time PCR (qPCR) assay

Specific primers are used to detect the expression of related genes in the kidney, liver, and gonads by RT-PCR. The reason for choosing the kidney and liver is because fish trunk kidney is rich in thyroid follicles (Geven et al. 2007) and liver is one of the THs-target tissues (de Oliveira et al. 2020). In short, Trizol is used to extract the total RNA of the tissue, and then the concentration and purity of the RNA are tested. RNA is reverse transcribed into cDNA using a reverse transcription kit. Then, we use LightCycler® 96 real-time fluorescent quantitative PCR system to detect related genes. Trizol, reverse transcription kit, and SYBR Green used were all purchased from Accurate Biotechnology (Hunan) Co., Ltd. With *β-actin* gene as the internal reference, the technique was repeated three times, and the relative gene expression was analyzed according to the formula of $2^{-\Delta\Delta CT}$. We examined the expression of several genes of thyroid follicles in trunk kidney: corticotropin-releasing hormone (*crh*), thyrotropin-releasing hormone (*trh*), thyroglobulin (*tg*), transthyretin (*ttr*), and the expression of special thyroid genes in the liver: type I deiodinase gene (*dio1*), type II deiodinase gene (*dio2*). And the genes related to the gonads are detected, including aromatase (*cyp19b*), follicle-stimulating hormone receptor (*fshr*), synthesis rapid regulator protein (*star*), and vitellogenin (*vtg*). The relevant information of the tested gene is listed in Table 1.

Data statistical assays

The data were presented as means ± standard error (SE). The difference between treatments and the control was tested using a one-way analysis of variance ANOVA with the software SPSS, and followed by Tukey test. The values of $p < 0.05$ (*) and $p < 0.01$ (**) were considered as significant and highly significant, respectively.

Table 1 Primers used for quantitative real-time PCR

Gene name	Sequence of the primers (5′-3′)
β-actin	*F: GGCTGTGCTGTCCCTGTA*
	R: GGCGTAACCCTCGTAG
crh	F: AACCACGAGCAAACCCATCA
	R: ACAGTTTTGCGCTTCATATACACC
trh	F: CAAGAGTGACTGGCGAAAGAG
	R: GTAGAAGCACCTAAGCCCTGA
tg	F: GAAGAAAGCAGCCAGTT
	R: CTCTGAGCCCTGACATT
ttr	F: TGGAGTTTGACACTAAAGCCTACT
	R: CCAGAGTGTAATGACGATGCC
dio1	F: CGCAGATTTCCTCATTGTTTACC
	R: CAAGCCTCTCCTCCAAGTTT
dio2	F: TGTCACTCCTGAGCTGTTCG
	R: TTGGAGTTTGGAGCAGAACAC
cyp19b	*F: CCAGTCGGTAGGTTGTTCCC*
	R: TGCAGGTTTACAGTGCAAGTTC
fshr	*F: CAGCTTGGTGTCCTTCACTCT*
	R: ACGTGCCAGCTTTGGATTGC
star	*F: GCAAGCTGTTACAAGGCTGATT*
	R: AGCTTGGCCCTGAAAAGAGT
vtg	*F: ATCCAAGGATCCCCCTCACA*
	R: ACACTGTTGCTCCAATTCCAT

Results

Effects of NOR on the hematocyte of fish

After 42 days of exposure to NOR, the number of red blood cells in the 1 mg/L NOR treatment group was significantly higher than that in the control group. However, there was no significant difference in the number of white blood cells between the experimental groups and the control group. The ratio of granulocytes in the 1 mg/L NOR treatment group was significantly lower than that in the control group. NOR exposure at 100 ng/L had no significant effect on the classification and count of white blood cells (Table 2).

Effects of NOR on the thyroid endocrine system of fish

Thyroid hormone levels

After NOR treatment, the levels of thyroid hormones (T4 and T3) in fish plasma were measured in all groups (Fig. 1).

Table 2 Parameters of hematocyte in carp following exposure to norfloxacin

Hemocyts	Unit	Control	100 ng/L	1 mg/L
Red blood cells	10^6 cell/mL	166.57 ± 17.50	154.83 ± 27.42	203.00 ± 32.44*
White blood cells	10^6 cell/mL	101.07 ± 38.62	100.40 ± 21.28	122.20 ± 17.58
Differential leukocyte count	Monocyte (%)	0.79 ± 1.37	1.01 ± 1.17	2.05 ± 1.51
	Lymphocyte (%)	49.87 ± 3.38	41.71 ± 10.47	60.81 ± 13.38
	Neutrophil (%)	48.84 ± 2.61	55.29 ± 10.03	34.52 ± 8.95*
	Monocyte (%)	1.29 ± 1.33	2.99 ± 2.00	4.68 ± 5.52

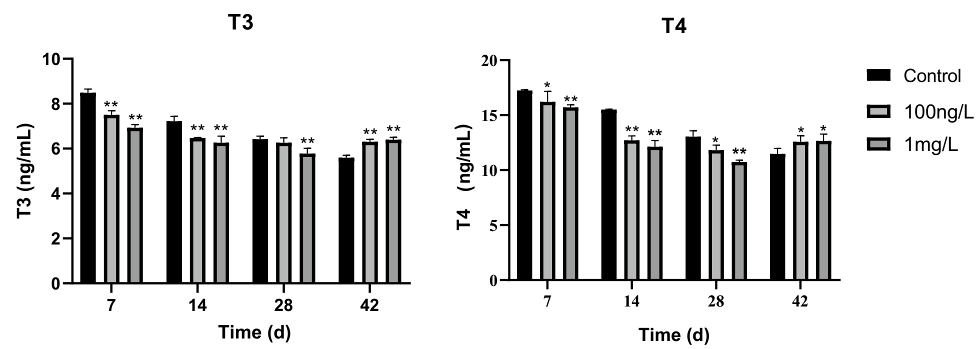

Fig. 1 The levels of T3 and T4 at different times in blood of common carp in each group. Data are means ± SEM ($n = 3$). Significant differences compared with control value; *$p < 0.05$, **$p < 0.01$

At 7, 14, and 28 days of exposure, T3 levels were significantly reduced in all treatment groups. However, a significant increase in T3 levels was detected in all exposure groups after NOR treatment for 42 days ($p < 0.01$). The levels of T4 showed similar trends to those of T3 in all tested fish.

Changes in the relative expression of thyroid-related genes

Figure 2 shows the expression levels of genes related to the HPT axis in juvenile common carp exposed to NOR for 42 days. In the HPT axis, compared with the control group, the expression of *crh*, *ttr*, *ttr*, which of thyroid follicles in trunk kidney and *ddio*, *dio2* genes of thyroid in the liver showed an increasing trend in the 100 ng/L NOR and 1 mg/L NOR treatment groups, but there was a significant increase in *crh* and *dio2* expression with the highest concentration of NOR, i.e., 1 mg/L ($p < 0.05$).

Effects of NOR on the gonad system of fish

Sex hormone levels

After NOR treatment, the levels of sex hormones (PROG and 11-KT) in fish plasma were measured in all groups (Fig. 3). At 7, 14, and 28 days of exposure, the levels of PROG and 11-KT increased significantly as NOR concentration increased; however, at 42 days of exposure to 100 ng/L NOR, PROG and 11-KT levels were significantly lower than those observed in the control group. Although a declining trend was observed in the 1 mg/L NOR treatment group at day 42, this change was not significant.

Changes in relative expression of gonad-related genes

Figure 4 shows the expression levels of genes related to the gonadal system in juvenile common carp exposed to NOR for 42 days. Exposure to NOR at 100 ng/L and 1 mg/L downregulated *Cyp19b* in gonadal tissues, but not significantly in comparison with control group expression levels. However, the transcription level of *fshr* decreased significantly (81.61-fold; $p < 0.01$) following 100 ng/L NOR exposure; by contrast, *fshr* expression increased in the 1 mg/L NOR treatment group but not significantly. Although expression levels of *star* did not differ significantly between the treatment and control groups, the transcription levels of *vtg* decreased significantly with 100 ng/L NOR exposure (8.69-fold; $p < 0.05$); contrastingly, a nonsignificant increase in *vtg* levels was observed in the 1 mg/L NOR treatment group.

Discussion

Effects of NOR on hematocyte parameters of fish

Blood parameter analysis plays an important role in evaluating the toxicity of xenobiotics to fish (Bojarski and Witeska 2020, Burgos-Aceves et al. 2019; Li et al. 2011). Exposure to antibiotics may cause changes in fish blood parameters including RBC and WBC. But there are few data on antibiotics. After being fed oxytetracycline for 56 days, the RBC and WBC content of *Oreochromis niloticus* decreased (Omoregie and Oyebani 2002). However, in our results, the number of red blood cells of carp in the 1 mg/L NOR group was significantly increased compared to the control group,

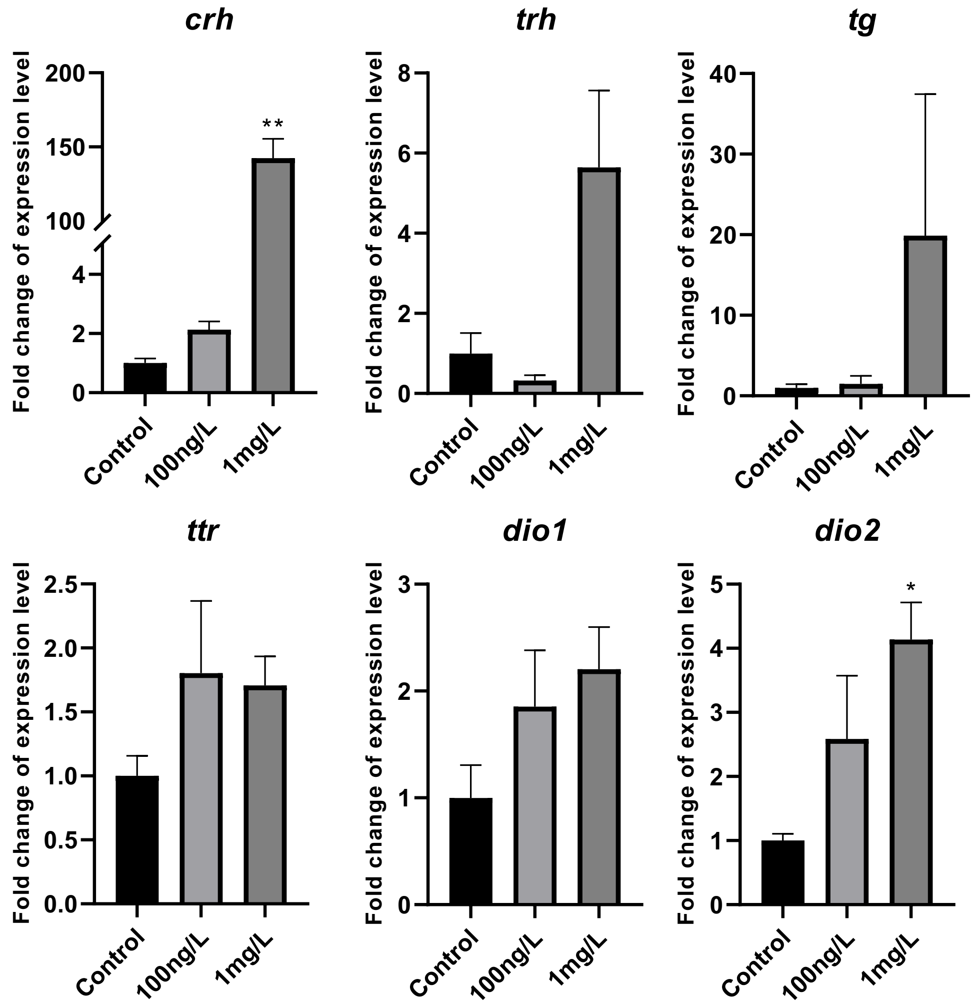

Fig. 2 The expression levels of genes involved in the HPT axis of juvenile common carp exposed to NOR for 42 days, including *crh*, *trh*, and *tg* in the kidney and *ttr*, *dio1*, and *dio2* in the liver. Data are means ± SEM ($n=9$). Significant differences compared with control value; $*p<0.05$, $**p<0.01$

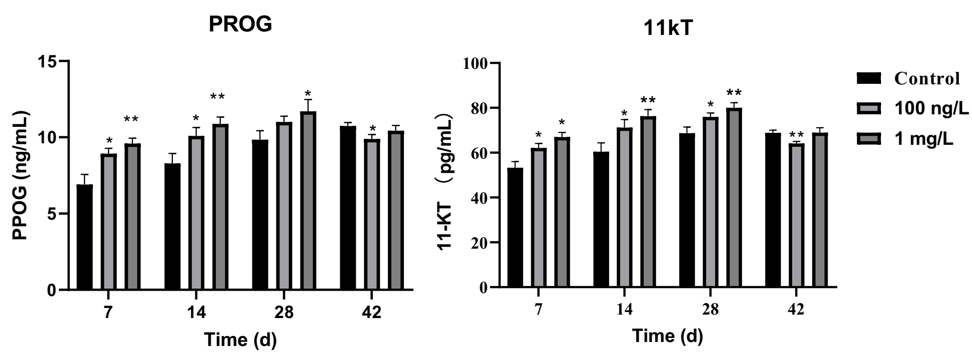

Fig. 3 The levels of PROG and 11kT at different times in blood of common carp in each group. Data are means ± SEM ($n=3$). Significant differences compared with control value; $*p<0.05$, $**p<0.01$

while the 100 ng/L NOR group had no significant change, which may be caused by NOR dose factors. In short, NOR may cause disturbances in the normal physiological state of carp blood. Changes in white blood cell count differences are considered to be sensitive indicators of environmental stress (Cole et al. 2001). Normally, in response to stressors, the number of lymphocytes decreases, and monocytes and neutrophils increase simultaneously (Murad and Houston 1988). Yonar reported that the use of OTC reduced the white blood cells, respiratory burst, and phagocytic activity of rainbow trout and increased serum protein (Yonar 2012). Dobšíková and others reported that OTC administration resulted in a decrease in the percentage of carp WBC, lymphocytes, and monocytes (DobÍková et al. 2013). Kasagala and Pathiratne reported that OTC treatment can lead to carp leukopenia, neutropenia, and reduced phagocytosis (Kasagala and Pathiratne 2008). Oxytetracycline can significantly inhibit the immune system of trout, which is characterized

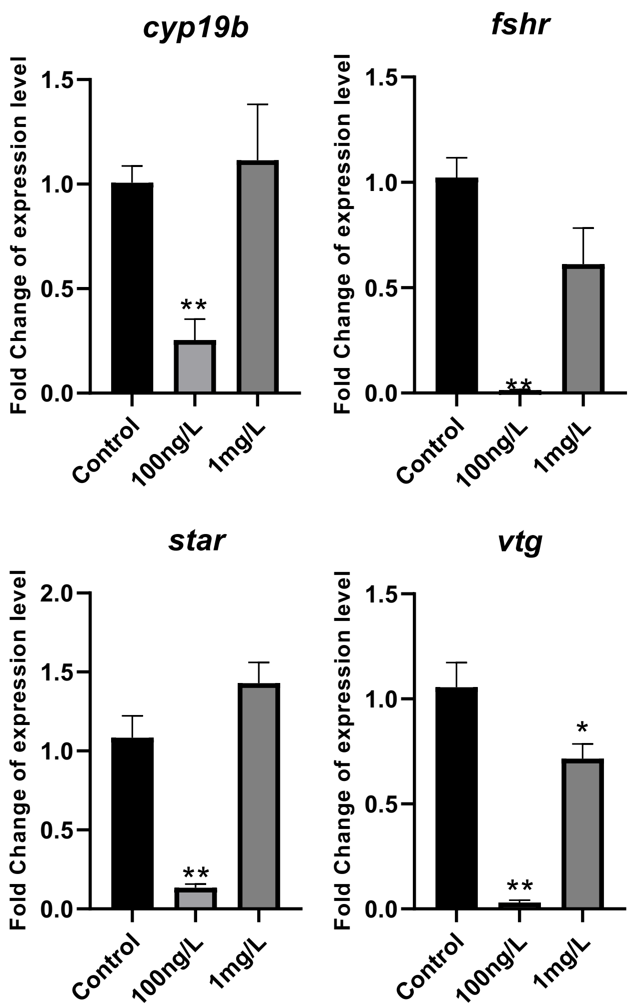

Fig. 4 The expression levels of genes involved in the gonad of juvenile common carp exposed to NOR for 42 days, including *cyp19b*, *fshr*, *star*, and *vtg*. Data are means ± SEM ($n = 9$). Significant differences compared with control value; $^{*}p < 0.05$, $^{**}p < 0.01$

by a significant decrease in serum lysozyme and ACH50 activity and total Ig levels, accompanied by leukopenia, neutropenia, and mononucleosis. However, in our research, WBC did not change significantly. However, neutrophils, as blood cells that produce lysozyme (Saurabh and Sahoo 2008), in the 1 mg/L treatment group was significantly lower than that in the control group. This result is consistent with previous studies.

Effects of NOR on hypothalamus–pituitary–thyroid axis in fish

Different environmental hormones can cause an imbalance in plasma thyroid hormone levels, and an increase or decrease in thyroid hormones can influence the physiological and metabolic processes of fish. This was confirmed in previous studies: low concentrations of triphenyltin were shown to cause dysregulation of the thyroid endocrine system in zebrafish (Li et al. 2019, 2020); mercury chloride was found to be toxic to the thyroid endocrine system of grass carp under certain temperatures (Li et al. 2021); and coexposure with butachlor and triadimefon was reported to affect the thyroid endocrine system of larval zebrafish (Cao et al. 2016).

In fish, thyroid follicles are the sites of thyroid hormone synthesis and storage, and the kidneys contain many thyroid follicles. Unlike mammals, fish rarely synthesize T3, but they do synthesize T4 mainly in their thyroid follicles. Most T3 comes from the deiodination transformation of T4 in the presence of deiodinase. However, T3 is the main active thyroid hormone type. In the present study, at 7-, 14-, and 28-day post-NOR exposure, T3 and T4 levels were significantly decreased in carp, which may have been because of the toxic effect of the antibiotic stimulating the juvenile carp to produce an environmental stress response at the early stage of exposure, which in turn inhibited the synthesis and secretion of thyroid hormone in plasma. After NOR exposure for 42 days, however, T4 levels increased in both NOR-treated groups relative to the control group, whereas T3 levels significantly decreased. The results are consistent with those reported in previous studies indicated that toxicants can result in the disruption of hormone levels (Kang et al. 2017; Li et al. 2014, Mishra and Mohanty 2015).

The synthesis and secretion of thyroid hormones (mainly T3 and T4) in the blood are affected by the expression of several genes in the HPT axis. We detected the expression of thyroid-related genes in NOR-exposed carp (Fig. 5); for example, the 42-day exposure increased the expression of *crh*, *trh*, and *tg* genes, which was consistent with the trend in T4 levels. This may have been because long-term exposure to NOR caused related genes to be activated by negative feedback regulation in the body. Iodothyronine deiodinase occupies a major position in regulating the secretion of fish. It effectively controls the intracellular and circulating levels of thyroid hormone. (Van der Geyten et al. 2005). In this study, *ttr*, *dio1*, and *dio2* gene expressions increased at varying levels, which was consistent with changes in blood T3 levels at 42 days. This showed that T4 iodine levels and the transformation process were enhanced, which in turn caused T3 content to rise in the blood and produce biological activity. Our results suggest that exposure to NOR induces thyroid disruption in fish in a concentration-dependent manner, but prolonged exposure can lead to the development of adaptive mechanisms that reduce the damage caused by the drugs.

Effects of NOR on hypothalamus–pituitary–gonad axis in fish

The reproductive system of fish is regulated by the HPG axis. Sex hormones play an important role in the regulation

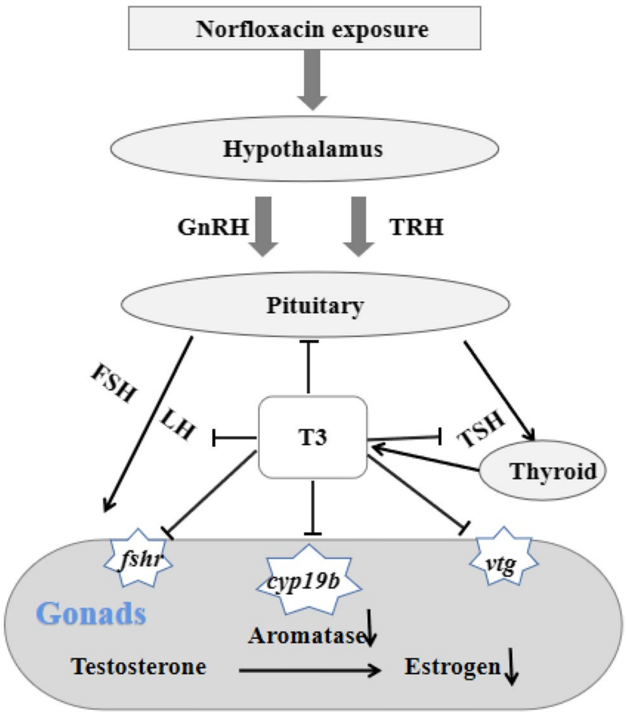

Fig. 5 The effect of T3 on the gonads. The production of pituitary gonadotropin, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) is stimulated by gonadotropin-releasing hormone (GnRH). LH and FSH stimulate gametogenesis and steroid production in the testes and ovaries. Aromatase converts androgens into estrogen

of fish gonadal differentiation, gametogenesis, and reproduction (Devlin and Nagahama 2002). We effectively evaluated the effect of NOR on the reproductive system of carp by detecting the sex hormones and the transcription levels of gonad-related genes.

In this study, it is interesting that hormone levels and gonadal gene changes after 42 days of exposure were significantly downregulated in the 100 ng/L NOR treatment group. This may be because aromatase (CYP19) plays an essential role in the process of estrogen synthesis, catalyzing the production of estradiol and estrone from androstenedione and testosterone. The increase or decrease of its expression level affects the rate of estrogen synthesis, which leads to metabolic imbalance in fish. At the same time, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) regulate the development and maturation of the gonads during the reproductive activities of organisms. They combine with the corresponding FSH and LH receptors to regulate the production of gametes in organisms. Generally, LH is secreted by the pituitary gland and combined with LH receptors, which stimulates follicular membrane cells around the follicle to produce androgens; aromatase then regulates FSH to convert androgens into estrogen. In the present results, the expression of *fshr* in the 100 ng/L NOR treatment group decreased significantly. In addition, vitellogenin (VTG) is a prerequisite for the production of vitellin, which provides nutrients/functional substances such as amino acids, fats, and vitamins for the development of fish embryos. The results of this study showed that although exposure to 1 mg/L NOR for 42 days increased *vtg* expression, exposure to 100 ng/L NOR for 42 days significantly reduced *vtg* gene expression in carp gonadal tissues. This result may be attributable to the level of estrogen induced at 100 ng/L NOR changed the expression level of *vtg*. In previous studies, endocrine disruptors can cause abnormal vitellogenin induction. For example, estrogen exposure can lead to decreased reproductive performance and decline in wild fish populations (Meijide et al. 2016). In the process of steroid hormone synthesis, steroidogenic acute regulatory (i.e., *star*) protein is an important rate-limiting factor that is mainly involved in the transport of cholesterol from the outer mitochondrial membrane to the vascular endothelium. In intima (Clark et al. 1996), the signal transduction of steroid hormone synthesis can cause the rapid expression of the *star* gene (Minegishiac 2003), which enhances the process of steroid hormone synthesis as well as increasing STAR protein expression. In our study, exposure to a range of NOR concentrations had no significant effect on *star* levels, despite an increasing expression trend in NOR treatment groups. We speculate that NOR intervention reduces the transport rate of cholesterol from the outer mitochondrial membrane to the inner membrane, but the effect may not be significant because of the low exposure concentration of NOR.

In short, after 42 days of exposure, 100 ng/L NOR will significantly inhibit the expression of carp and sex hormones and related gonadal genes. Interestingly, genes expression in the 1 mg/L NOR treatment group showed a tendency to recover. It is likely that carp develop resistance to prolonged drug exposure, which explains the lack of a significant change.

The relationship between HPT and HPG axis crosstalk in fish

Another interesting aspect worthy of discussion is the intersection of the HPT and HPG axes (Fig. 5). The expression and activity of gonadal aromatase (CYP19) are important for estrogen production because they help convert androgens into estrogen. Treatment with T3 in vivo can reduce the level of *CYP19* mRNA in goldfish testes, and a similar reduction has been observed in goldfish ovaries (Nelson et al. 2010). Additionally, studies have shown that thyroid hormones can weaken the reproductive axis of goldfish by reducing the expression of gonadal aromatase and further reducing the synthesis of estrogen. These findings are consistent with our research results; the T3 content of the treatment group increased relative to that in the control group, whereas CYP19b levels tended to decrease.

Thyroid hormones also have effects on the maturation of the gonads of bony fish, i.e., they affect the initiation of spermatogenesis in males and the growth of follicles formed by the yolk of females (Cyr and Eales 1996). T3 can induce an increase in mature sperm (Lema et al. 2009), and exposure to T4 is known to cause premature spermatogenesis in juvenile carp (Timmermans et al. 1997). Thyroid hormone effects are dose dependent in female rainbow trout, e.g., physiological levels of T3 promote ovarian growth, whereas higher levels inhibit ovarian growth (Cyr and Eales 1988). In the African clawed frog (*Xenopus laevis*), T3 enhances estradiol-induced vitellogen activation (Ulisse and Tata 1994); an increase in *vtg* mRNA also indicates that thyroid hormones play roles in frog ovarian maturation.

Nelson et al. reported that when T3 was injected into the goldfish body, the pituitary *LHβ* transcription level was significantly reduced, and the *FSHβ* transcription level also decreased, but it did not reach a statistically significant level (Nelson et al. 2010). Generally, the decrease of LH and FSH in fish will not completely inhibit reproduction, but it will inhibit the reproduction and reproduction by reducing the level of LH-mediated hormone production and the ability to induce gametogenesis. Howland et al. also found that T3 treatment can reduce the circulating levels of LH in rats (Howland and Ibrahim 1973), which suggests that the damage produced by thyroid hormones to LH may be a common phenomenon in vertebrate species. Similarly, when the T3 level of the 100 ng/L NOR treatment group increased compared to the control group, a decrease in *fshr* expression was found.

In this study, the changes in gene expression or hormone levels involved in the HPG axis and the HPT axis occurred at the same time, although our results are consistent with previous studies on the crosstalk between the HPG axis and the HPT axis. Whether the changes in the HPG axis are secondary to the disorder of the HPT axis caused by norfloxacin exposure, or the disorder of the HPG axis and the HTP axis at the same time, needs to be further explored in the future.

Conclusion

In conclusion, in this study, it was mainly found that after 42 days of exposure, the number of red blood cells of carp in the 1 mg/L NOR group increased significantly compared with the control group. The significant decrease in the number of granulocytes may be due to the immunosuppression of carp caused by antibiotics. In addition, after 42 days of NOR exposure, compared with the control group, the T4 and T3 levels of the two NOR treatment groups increased on average, which was consistent with the changes in the trend of genes related to the HPT axis. This indicates that the T4 iodine level and the conversion process are enhanced, which in turn leads to an increase in the T3 content in the blood and biological activity. Moreover, 100 ng/L NOR can inhibit the level of sex hormones and inhibit the expression of related genes in the HPG axis. The change in 1 mg/L NOR does not seem to be obvious. This may be that long-term exposure exceeding a certain concentration range can lead to the development of adaptive mechanisms. Nevertheless, we cannot ignore the risks of quinolone antibiotics to aquatic animal reproduction and population health. Although some research exists on endocrine toxicity related to environmental factors, quinolone antibiotics have rarely been studied in this context. Moreover, the interaction between thyroid function and gonadal development in bony fish is not fully understood. The data obtained in the present study, however, can provide a theoretical basis on which quinolone antibiotic-related ecotoxicological research can be conducted in aquatic organisms to develop appropriate monitoring and early warning strategies.

Author contribution Siqi Zhang: writing—original draft preparation, focused on HPT analysis; Xueli Zhao: methodology and software, focused on HPG analysis; Shuwen He: indices measurement; Shaoying Xing: data analysis; Zhihan Cao: fish culture and sampling; Ping Li: writing—reviewing and editing, guide the HPG analysis; Zhihua Li: conceptualization and overall guidance, and guide HPT analysis.

Funding This work was financially supported by the Natural Science Foundation of Shandong Province, China (No. ZR2019MC011), National Key Research and Development Program of China (2018YFD0900905, 2018YFD0900902).

Data availability The data of this paper are from our experiment and based on the previous published papers cited in our paper.

Declarations

Ethics approval This manuscript is ethical.

Consent to participate and consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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