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RESEARCH ARTICLE

Stereoselective effects and its potential mechanism of ofloxacin on the growth and development of *Rana nigromaculata*: mainly liver lipid metabolism

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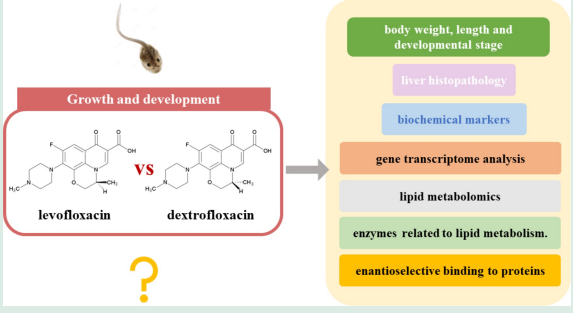
HIGHLIGHTS

- Ofloxacin has stereoselective inhibitory effect on tadpole growth and development.
- Inhibition effect of ofloxacin on tadpole development can last for 75 d.
- Differential genes between ofloxacin and its enantiomer are mainly immune-related.
- Ofloxacin and its enantiomer show the potential to promote obesity.
- Two enantiomers were selectively bound to enzymes related to lipid metabolism.



ABSTRACT: Most research on antibiotics in the environment disregards chiral antibiotics, such as ofloxacin (OF). In this study, tadpoles of *Rana nigromaculata* were exposed to 1 μg/L OF and levofloxacin (LVFX, an enantiomer of OF) for 75 d. Compared with dextrofloxacin, LVFX treatment had a greater effect on the inhibition of bodyweight, body length, development stage, and pathological liver damage. Therefore, OF exerts a stereoselective inhibitory effect on both growth and development, which is consistent with the results at the systemic metabolism level. Transcriptomic analysis revealed that the differentially expressed genes between OF and LVFX were mainly immune related. Targeted metabolomics showed that the stereoselective biological effect of OF on *R. nigromaculata* was caused by differences in contents of PE-O 16:0–22:4, PE 16:0–14:0, TAG 45:0–FA16:0, PE-P 18:0–16:0, PE 16:0–16:0, PC 16:0–22:4 + AcO, PE 18:1–18:3, PC 16:1–18:1 + AcO, and PC 18:1–18:3 + AcO. Furthermore, two enantiomers of OF were selectively bound to enzymes related to lipid metabolism. This study provides both theoretical and practical references for the accurate evaluation and scientific control of the ecological risk of chiral antibiotics.

KEYWORDS: Chiral antibiotics, Amphibians, Targeted metabolomics, Molecular docking, Obesity

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1 Introduction

Quinolones are among the most widely used antibiotics in aquaculture because of their broad antibacterial spectrum, strong antibacterial activity, and lack of cross-resistance with other antibiotics (Han et al., 2021; Ren et al., 2021; Zhang et al., 2023b). In the medical field, quinolones are a very important class of anti-infective drugs (Chen et al., 2024). However, studies have shown that these antibiotics can affect the growth, reproductive capacity, health and behavior of aquatic organisms (Zhu et al., 2016; Feng et al., 2018). In addition, these antibiotics may be transmitted through the food chain, posing a potential threat to higher-level organisms. Eventually, the ecological balance of aquatic organisms will be disrupted, which will negatively impact the aquatic ecosystem (Zhang et al., 2020; Kergaravat et al., 2021). Ofloxacin (OF) is a quinolone antibiotic, which easily accumulates in the aquatic environment because of its environmental persistence (Zhou et al., 2020). A study examined the levels of three fluoroquinolones in wastewater from the Kahuta and Hattar industrial areas in Pakistan. The median concentration of levofloxacin (LVFX), an enantiomer of OF, was measured in wastewater samples from both industrial areas. The authors found levels as high as 36.7 μg/L in Kahuta samples, while the concentration of LVFX in samples from Hattar industrial area was even higher, at 48.9 μg/L (Riaz et al., 2017). The highest concentrations of LVFX detected in Japanese and Chinese effluents were 3600 and 6800 ng/L, respectively (Ghosh et al., 2016). The concentration of LVFX detected in Portuguese effluents ranged from 34 to 438 ng/L (Cristóvão et al., 2020).

At present, most research on antibiotics in the environment ignores the important fact that certain antibiotics are chiral compounds, such as OF. Previous studies have shown that different enantiomers of chiral drugs may have significantly different toxicity to organisms (Sanganyado et al., 2017; Arenas et al., 2022). Therefore, the enrichment and metabolism of different enantiomers of chiral antibiotics in non-target organisms need to be analyzed separately. Moreover, their potential impacts on the ecological environment and human health need to be re-evaluated to truly and objectively reflect the ecological risks of chiral antibiotics (Kan et al., 2023).

The recent decline in the number of amphibians across the world has caused scholars to explore the underlying reasons (Lips et al., 2008). Luedtke et al. (2024) reported the results of the second global amphibian assessment, which assessed 8011 species of amphibians for the Red List of Endangered Species of the International Union for Conservation of Nature. They found that 40.7% of amphibians were threatened globally, identifying them as the most threatened category of vertebrates. There are many reasons for this decline of amphibians, such as the devastating epidemic caused by pitcher plants, the loss and degradation of habitats, global warming, and chemical pollutants (Stuart et al., 2004).

The developmental stage of amphibians from larvae to mature individuals is inseparable from the aquatic environment, especially for the tailless amphibian larvae, also known as tadpoles (Guo et al., 2022). Therefore, it is necessary to evaluate the effects of antibiotics on amphibians. Following exposure to LVFX for 28 d, researchers found that the proteins related to thyroid development were affected in tadpoles of *Rana nigromaculata*. Affected proteins were deiodinase and thyroid stimulating hormone, which led to developmental inhibition in *R. nigromaculata* tadpoles (Zhang et al., 2023a).

Recent studies have found that environmental pollutants can interfere with both lipid metabolism and energy balance, thus causing lipid metabolism disorders (Blanc et al., 2021; Hamid et al., 2021). Once the lipid metabolism is imbalanced, serious metabolic diseases can emerge (Hennig et al., 2012). Therefore, understanding the impact and mechanism of environmental pollutants on lipid metabolism is of great importance for comprehensive assessments of the health risks of environmental pollutants. The liver is the main organ that absorbs most foreign substances after exposure (Petersen et al., 2017; Wang et al., 2021). In addition, the liver plays a central role in the regulation of physiological, digestive, and metabolic processes (such as fat synthesis and oxidation, bile secretion, and detoxification). Although studies have reported adverse effects of OF on amphibian tadpoles, very little information is available on the molecular mechanism of lipid metabolism in the livers of amphibians exposed to OF.

In this study, *R. nigromaculata* tadpoles were exposed to 1 μg/L OF and LVFX. After 75 d of exposure, the bodyweight, body length, and developmental stage were assessed. Then, the liver histopathology was analyzed, the biochemical metrics associated with lipid metabolism were determined, and a quantitative proteomics analysis of different substances in systemic lipid metabolism was conducted. To further explore the mechanism, gene transcriptome analysis and drug-protein molecular docking were conducted. The aim of this study was to explore the stereoselective effect of OF on the liver lipid

metabolism of *R. nigromaculata* larvae and its underlying mechanism. This study provides both theoretical and practical references for the accurate evaluation and scientific control of the ecological risk posed by chiral antibiotics.

2 Materials and methods

2.1 Chemicals

OF (CAS: 82419-36-1; purity = 99%) and LVFX (CAS: 100986-85-4; purity ⩾ 98%) were provided by Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China. Stock solutions of OF or LVFX with concentration of 500 μg/L were prepared by adding dimethyl sulfoxide.

2.2 Maintenance of tadpoles and exposure

R. nigromaculata tadpoles at Gosner stages 25–26 were obtained from the Eco-Environmental Research Center of the Chinese Academy of Sciences (Gosner, 1960). Tadpoles were cultivated under the conditions described in a previous study (Zhang et al., 2023a).

Fifty healthy tadpoles with the same size and development stage (Gosner stage 26) were randomly selected and placed into an aquarium containing 20 L exposure solution with stock solutions of OF or LVFX as well as dechlorinated water. In the treatment groups, exposure solutions reached concentrations of 1 μg/L OF or LVFX (near the environmental detection level). The exposure solution in the control group was an equal volume of dimethyl sulfoxide added to dechlorinated water. Three replicates were set for both the control and treatment groups. Exposure solutions were replaced every three days, and the replacement procedure used total water replacement. The culture conditions of the exposure experiment were as described above. After 75 d of exposure, tadpoles were anesthetized with MS-222, rinsed with distilled water, and dried with sterile paper towels; then, their weight, length, and developmental stages were recorded. Finally, the liver was dissected and weighed. The experimental processes strictly followed Institutional Ethical Guidelines under the approval number IEC-AUS/2015-032.

2.3 Liver histological examinations

After the liver samples were collected, dehydrated, sectioned, and stained, liver sections were observed under an optical microscope. All details about this assay are described in Supplementary material 1.1.

2.4 Analysis of biochemical markers related to lipid metabolism

Whole tadpoles (n = 3) in each treatment group were used for analysis. Total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol, free fatty acids, hepatic glycogen, and insulin were determined using corresponding ELISA kits (MIbio Company, China).

2.5 RNA extraction, library preparation, and Illumina Hiseq Sequencing

RNA from tadpoles was extracted and analyzed according to a previous study (Zhang et al., 2023a).

2.6 Lipid metabolomics

In this study, lipids of whole tadpoles from different treatment groups were assessed and missing values were substituted by half of the minimum value. The final data set containing information on compound name, sample name, and concentration was imported into the SIMCA16.0.2 software package (Sartorius Stedim Data Analytics AB, Umea, Sweden) for multivariate analysis. The procedure was based on a previous report (Chen et al., 2022).

2.7 Determination of proteins related to lipid metabolism

Whole tadpoles (n = 3) from each treatment group were used for analysis of proteins related to lipid metabolism. Fatty acid synthase (FAS), acetyl CoA carboxylase (ACC), hormone sensitive triglyceride lipase (HSL), lipoprotein lipase (LPL), and hepatic lipase (HL) were determined using corresponding ELISA kits (MIbio Company, China).

2.8 Molecular docking

To analyze the binding situation of S/R-ofloxacin and proteins related to lipid metabolism, molecular docking experiments were conducted according to our previous report (Zhang et al., 2023a).

3 Results and discussion

3.1 Growth and development of tadpoles

As shown in Fig. 1, the weight of tadpoles in the LVFX treatment group (0.597 ± 0.066 g) was significantly

lower than that of tadpoles in the control group (0.792 ± 0.068 g). Compared with the control (29.9 ± 1.3 mm), the SVL of tadpoles in LVFX and OF treatment groups increased significantly, at 34.8 ± 0.8 and 36.0 ± 0.3 mm, respectively. This increase may be attributed to the tadpoles in the control having developed to the tail absorption stage. According to the results of the development stage, LVFX has a stronger influence on the growth and development of tadpoles than OF. Compared with the control and OF treatment groups, fewer tadpoles were older than stage 40, but more tadpoles reached stages 37–39 in the LVFX treatment group.

Previous studies confirmed the effect of OF on the growth and development of *R. nigromaculata*. After 28 d of exposure, compared with the control group (1.105 ± 0.102 g), the weight of tadpoles in LVFX and OF treatment groups was significantly lower, at 0.494 ± 0.024 g and 0.813 ± 0.020 g, respectively. Moreover, OF exerts a stereoselective inhibitory effect on the growth and development of *R. nigromaculata*. This finding implies that the inhibitory effect of OF on the growth and development of *R. nigromaculata* began at the early stage of development (28 d). Furthermore, the inhibitory effect did not disappear with increase in exposure time.

3.2 Histological analysis

The results of histological analysis are shown in Fig. 2. In the control group, hepatocytes were evenly distributed, with clear cord structure, round nucleus in the center of cells, complete cell membrane structure, clear liver plate structure, and normal hepatic sinusoids, distributed among hepatocytes. However, in the OF treatment group, the structure of the hepatic plate became blurred, the vacuoles of cells increased (black arrow), the space between hepatic sinuses widened,

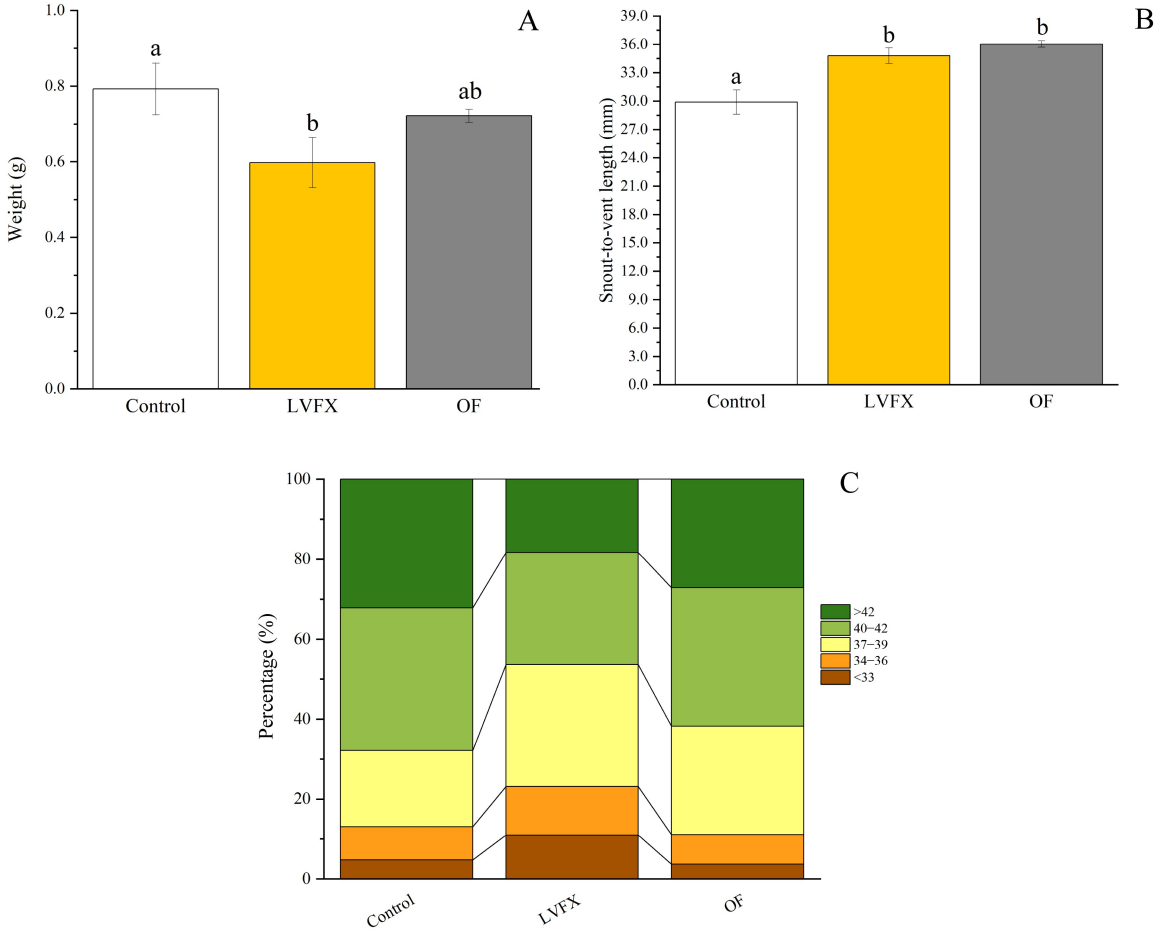

Fig. 1 Weight (A) and SVL (B) of tadpoles after 75-d exposure. (C) Development stages of tadpoles after 75-d exposure. ab: different letters represent statistically significant differences among treatments (SNK) at $P < 0.05$. Error bars indicate the standard deviation.

certain nuclei migrated to the outside of the liver, and several nuclei were shrunk, deformed, and dissolved (red arrow). Exposure to LVFX significantly aggravated the damage of the liver structure, showing loose cell arrangement, enlarged gaps, disappearance of cord structure, continuous enlargement of cell vacuoles, large-scale nuclear atrophy, dissolution and cytolysis, as well as necrosis of local tissues (red ellipse). Analysis results and statistics of liver histopathology are given in Table S1.

Related studies showed that under stress, the energy supply of young fish is mainly from the decomposition of liver glycogen. The vacuoles in liver cells might be due to the rapid consumption of energy by young fish, leading to the decomposition of liver glycogen or fat, resulting in vacuolation of liver cells (Kou et al., 2020; Yuan et al., 2023). The results of the present study are consistent with these findings in fish. Under the stresses of OF and LVFX, *R. nigromaculata* consumes a certain amount of energy to enable the stress reaction. Therefore, the energy of *R. nigromaculata* is consumed rapidly, which increases the physiological burden of the liver, leads to the decomposition of glycogen or fat, and finally results in the vacuolation and disorder of liver cells. Moreover, LVFX caused more pathological damage to the liver than OF, which is composed of half LVFX and half dextrofloxacin. Thus, it can be inferred that LVFX exerts stronger effects on the liver than dextrofloxacin.

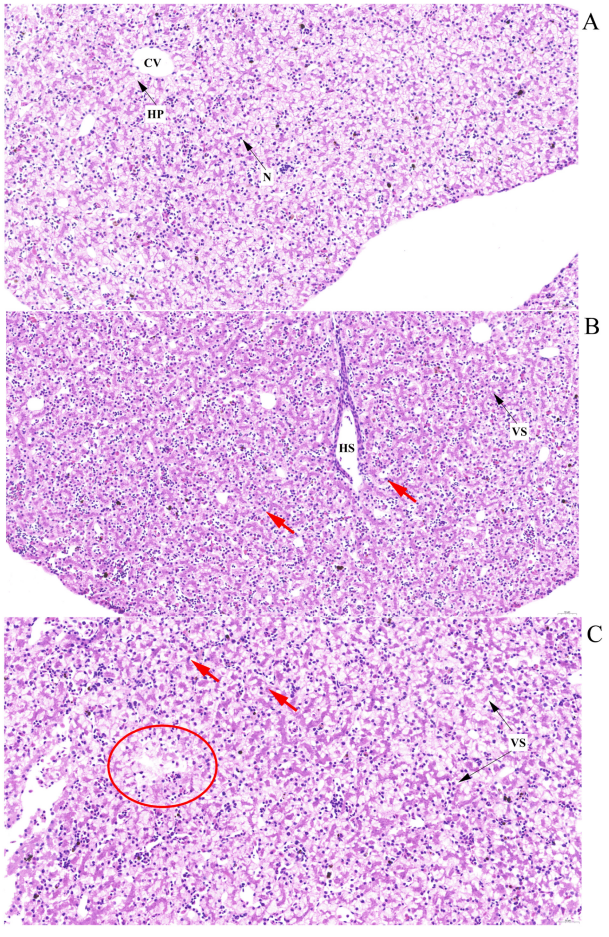

Fig. 2 Liver histopathology. (A) Control, (B) OF treatment, (C) LVFX treatment. CV, central vein; HP, liver plate; HS, hepatic blood sinusoids; VS, vacuole; N, nuclei; red arrows indicate cell nucleus pyknosis and nuclear dissolution; red ovals indicate local tissue necrosis and cavity formation.

3.3 Levels of biochemical markers related to lipid metabolism

The liver is the organ with the highest sensitivity to foreign substances, and it is also the main organ that controls the homeostasis of lipid metabolism (Wang and Tontonoz, 2018). Metabolic disorders are an important cause of liver disease. Exposure treatment inevitably affects the lipid metabolism by affecting the liver. To further explore the stereoselectivity of ofloxacin on lipid metabolism, biochemical markers related to lipid metabolism were analyzed. There was no significant difference in total cholesterol level between LVFX treatment group and control group, but a significant difference was observed between OF treatment group and control group (Fig. 3). LVFX and OF exerted stereoselective effects on the total cholesterol level of *R. nigromaculata*. To a certain extent, these effects can explain the inhibitory effect of dextrofloxacin on total cholesterol. The low total cholesterol level may be due to the fact that OF causes gastrointestinal malabsorption, liver disease, hyperthyroidism, anemia, or other consumptive diseases in *R. nigromaculata*; these diseases may interfere with the metabolism of nutrients or the ability of the liver to synthesize nutrients (Du et al., 2023). As the liver is the main organ for the synthesis and storage of total cholesterol, OF may interfere with the ability of the liver to synthesize nutrients. This is consistent with the liver weight of LVFX and OF groups being lower than that of the control group (on average). LVFX and OF exert stereoselective effects on the triglyceride levels of *R. nigromaculata*. LVFX significantly decreased the triglyceride level of *R. nigromaculata*, while OF significantly increased the triglyceride level, which can explain the triglyceride promotion of dextrofloxacin to a certain extent. The average level of high-density lipoprotein cholesterol for LVFX and OF treatment groups was higher than that of the control group. Furthermore, the difference between LVFX treatment group and control group was significant, but

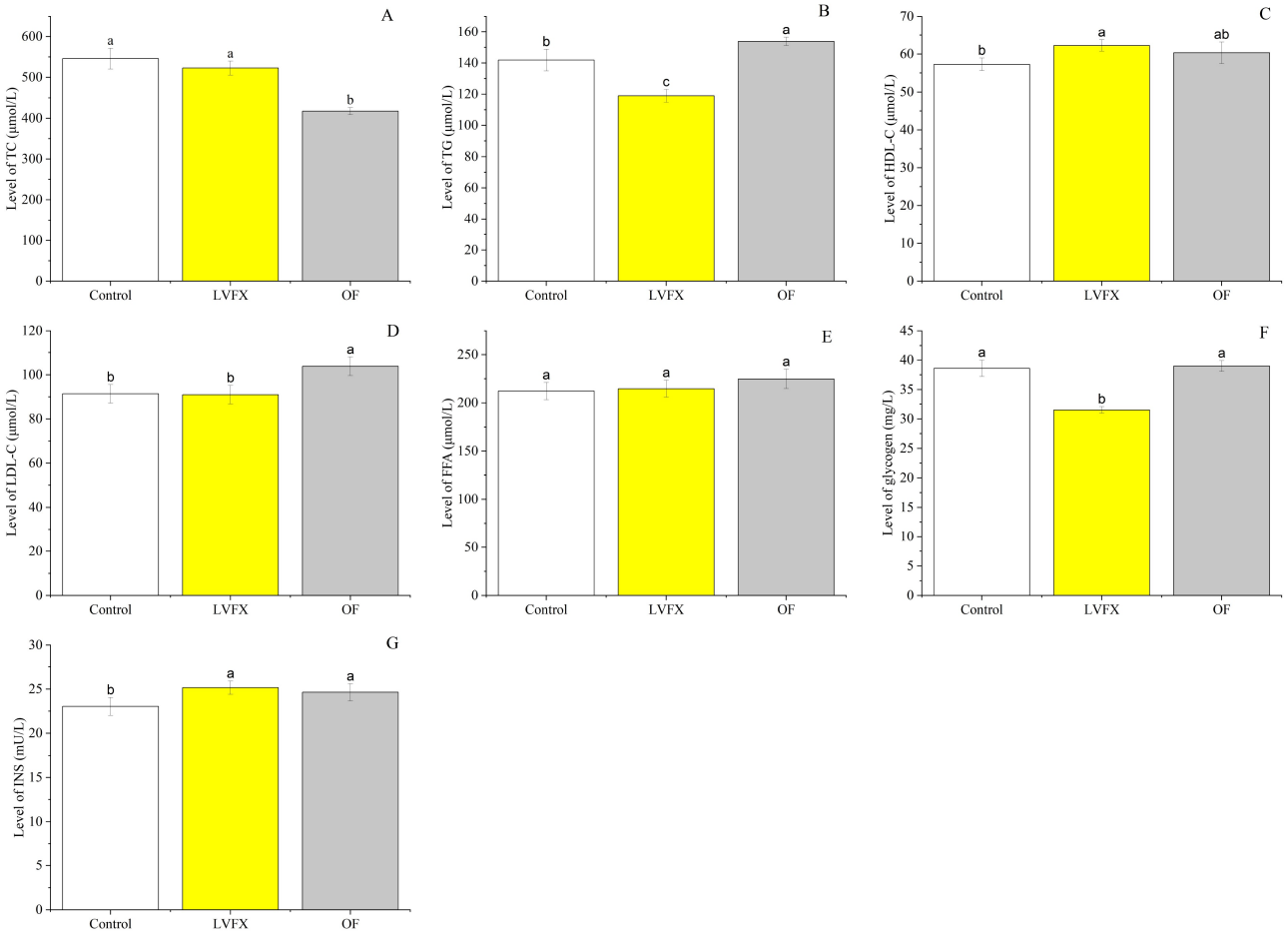

Fig. 3 Levels of TC (A), TG (B), HDL-C (C), LDL-C (D), FFA (E), HG (F) and INS (G) in tadpoles after 75-d exposure. Total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), free fatty acids (FFA), hepatic glycogen (HG) and insulin (INS). abc: different letters represent statistically significant differences among treatments (SNK) at $P < 0.05$. Error bars indicate the standard deviation.

no significant difference was found between OF treatment group and control group and LVFX treatment group. This result shows that LVFX and OF exert no stereoselective effect on the level of high density lipoprotein cholesterol in *R. nigromaculata*. LVFX and OF have stereoselective effects on the level of low-density lipoprotein cholesterol in *R. nigromaculata*. Specifically, LVFX has no significant effect on the low-density lipoprotein cholesterol level of *R. nigromaculata*, but OF significantly increases the level of low-density lipoprotein cholesterol. This finding can explain the promotion effect of dextrofloxacin on low-density lipoprotein cholesterol to a certain extent. Exposure treatment has little effect on free fatty acids and the results almost match those of the control group. It can be speculated that the concentrations of LVFX and OF used in the experiment are sufficiently low to have a significant impact on free fatty acids. Moreover, the mechanism of action of LVFX and OF is complex, involving multiple metabolic links and multiple targets in organisms. In this case, even under drug exposure, it may not have a significant effect on free fatty acids. OF has little effect on liver glycogen and shows no difference with the control group. However, LVFX has a strong effect on liver glycogen and shows a clear difference with the control group. Levofloxacin is the levoisomer of OF, and this structural difference may lead to their different affinities with different targets in organisms, thus affecting both their efficacy and side effects (Kaziem et al., 2020; Petrie and Camacho-Munoz, 2021). Subtle differences in the mechanism of action between LVFX and OF may lead to different effects on the hepatic glycogen metabolism. In addition, there may be differences in the absorption, distribution, metabolism, and excretion of these two drugs in organisms (Ribeiro et al., 2020). These differences may affect their concentration and residence time in target organs (such as the liver), thus indirectly affecting the

level of hepatic glycogen. Compared with the control, exposure to both LVFX and OF significantly increased insulin levels, which may be due to the following reasons. Both LVFX and OF are fluoroquinolones, which may increase insulin levels by affecting insulin secretion or the sensitivity of insulin receptors (Althaqafi et al., 2021; Juhl et al., 2023). These drugs may promote insulin secretion by pancreatic β cells, thus increasing the insulin level in the serum.

In summary, at the level of systemic metabolism, LVFX increased the levels of high-density lipoprotein cholesterol and insulin and decreased the levels of hepatic glycogen and triglyceride. Moreover, the influence of OF on *R. nigromaculata* was weaker than that of LVFX, which may be due to the effect of dextrofloxacin. Dextrofloxacin resulted in differences in indicators of the systemic metabolism level, such as increase in the levels of triglyceride, low-density lipoprotein cholesterol, and insulin in *R. nigromaculata*.

3.4 Transcriptome analysis

The number of differentially expressed genes between treatments is shown in Fig. S1. The GO enrichment results are shown in Fig. S2. The differentially expressed genes between OF and LVFX treatments are mainly genes involved in cell periphery and extracellular region functions, as well as genes involved in the immune system process, immune response, regulation of the immune system process, cytokine-mediated signaling pathway, and positive regulation of the immune system process. The enrichment degree of differentially expressed genes is shown in Fig. S3, where the greater the Rich factor, the greater the enrichment degree. More different genes have the functions of immune response-regulating cell surface receptor signaling and cytokine−mediated signaling.

KEGG enrichment results are shown in Fig. S4. The differently expressed genes between OF and LVFX treatments are mainly involved in cell adhesion molecules, tumor necrosis factor signaling pathway, cytokine-cytokine receptor interaction, viral protein interaction with cytokine and cytokine receptor, NF-kappa B signaling pathway, IL-17 signaling pathway, protein digestion and absorption, and mucin type O-glycan biosynthesis. As seen from Fig. S5, there are more differently expressed genes with the functions of mucin type O-glycan biosynthesis and viral protein interaction with cytokine and cytokine receptor.

The differentially expressed genes between OF and LVFX are mainly enriched in immune-related pathways. In recent years, lipid metabolism has been found to be closely related to immune function. Cytokines are a kind of small molecular protein with wide biological activities, which are synthesized and secreted by immune cells (such as monocytes, macrophages, T cells, B cells, and NK cells) and certain non-immune cells (endothelial cells, epidermal cells, and fibroblasts). B cells play an important role in lipid metabolism. The formation of FAS and fat metabolism enzymes decreases when the expression of B cell lymphoma 6 is down-regulated in the liver, fatty, and other tissues in mice. It can regulate the lipid metabolism by mediating the secretion of growth hormone, reducing fat production, and increasing fat decomposition (LaPensee et al., 2014). Type 1 NKT cells can significantly reduce the increase in adipose tissue and insulin resistance in mouse and human adipose tissue (Ji et al., 2012). Kotas et al. reported that triglyceride in the liver of CD1d knockout mice was clearly aggregated, while at the same time, the resistance of the liver to insulin could be increased by a high-fat diet; therefore, NKT cells in the liver could properly regulate the generation of lipids (Kotas et al., 2011). In case of overnutrition, macrophages accumulate in adipose tissue, thus transforming from an activated M2 phenotype to another classic M1 phenotype, which proves that excessive lipid intake can enhance the inflammatory response signal pathway (Jantsch et al., 2014). In summary, it can be speculated that the difference in immune-related pathways between OF and LVFX is due to the different effects of OF and LVFX on lipid metabolism. Therefore, at the gene level, OF and LVFX exert different effects on lipid metabolism.

3.5 Targeted lipid metabolomics

The lipid differential metabolites were analyzed between treatment groups (Fig. S6). The results are shown in Fig. 4. In the comparison between control and OF treatment groups, PE (30.77%), PI (23.08%), TAG (15.38%), and PG (15.38%) were the main differentially expressed components. The differences in their unsaturated bonds and carbon chain lengths are shown in Fig. S7. The control groups showed more unsaturated bonds than the OF treatment group, and the carbon chain of metabolites in the control exceeded that in the OF treatment group. The volcano plot shows the differences in metabolic substances between control and OF treatment groups from a statistical perspective. Correlation analysis was conducted on the 13 lipid metabolites with significant differences (Fig. S8) and the results showed that PI 18:1–24:4 had the highest correlation with other lipid metabolites.

In the comparison between CK and LVFX treatment

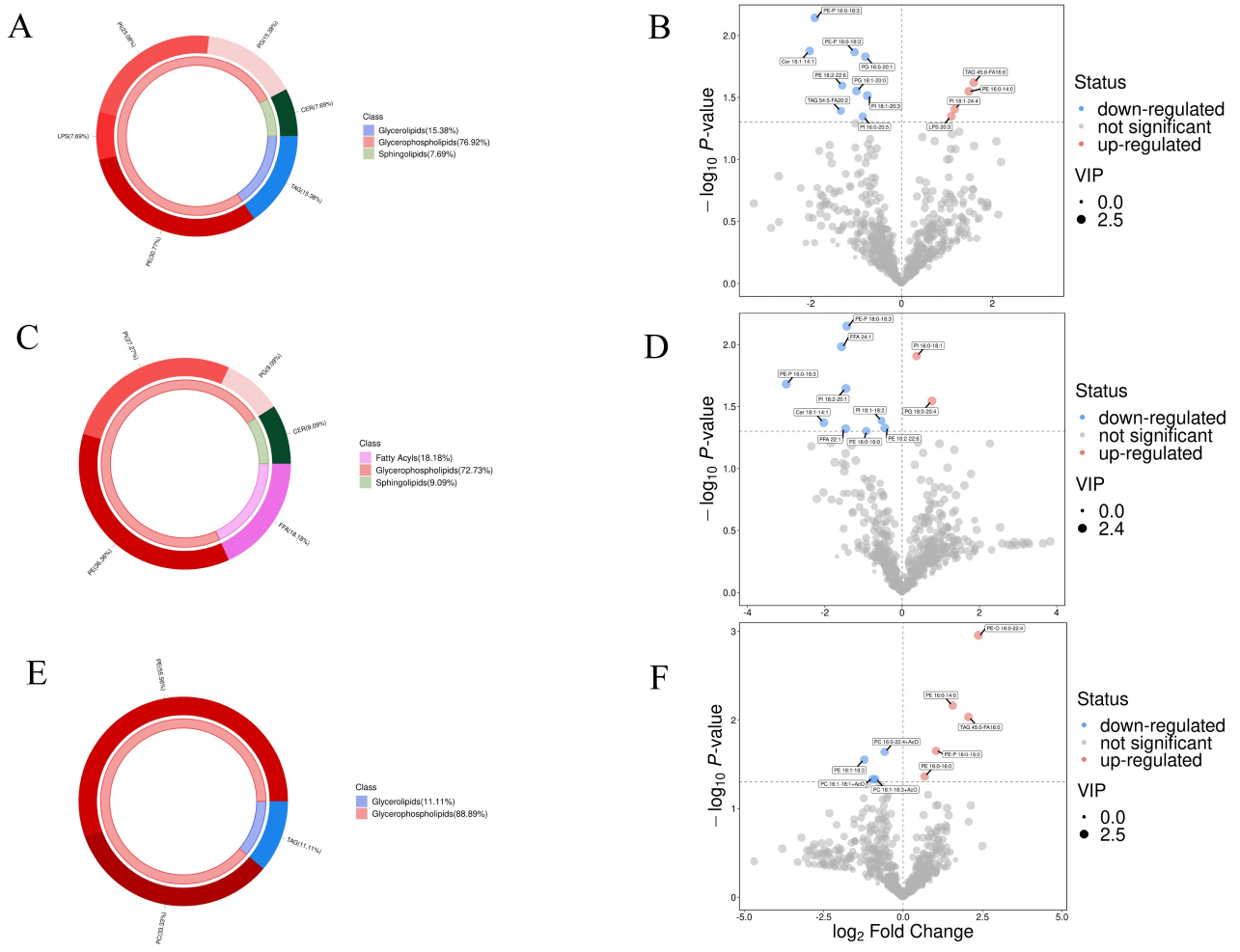

Fig. 4 Donut chart and Volcano plot of differential metabolites for group CK vs OF (A and B), CK vs LVFX (C and D) and LVFX vs OF (E and F), respectively.

groups, PE (36.36%), PI (27.27%), and free fatty acids (18.18%) were the main differentially expressed components. Information on the differences in their unsaturated bonds and carbon chain lengths is shown in Fig. S7. The control group contained more unsaturated bonds than the LVFX treatment group, and the carbon chain of the metabolites in the control was longer than that of the LVFX treatment group. The volcano plot presents the differentially expressed metabolites between the CK and LVFX treatment groups from a statistical perspective. Correlation analysis was conducted on the 11 lipid metabolites with significant differences (Fig. S8), and PE-P 16:0–18:3 and PE-P 18:0–18:3 had the highest correlation with other lipid metabolites. According to the volcano plot, compared to the control, both OF and LVFX exposure treatments significantly up-regulated PE-P 16:0–18:3, PE-P 18:0–18:3, Cer 18:1–14:1, and PE 18:2–22:6. These four substances may be stress response biomarkers in *R. nigromaculata* exposed to exogenous substances.

In the comparison between LVFX and OF treatment groups, PE (55.56%), PC (33.33%), and TAG (11.11%) were found to be the main differentially expressed components. The differences in their unsaturated bonds and carbon chain lengths are shown in Fig. S7. In PE components, there are more unsaturated bonds in the OF treatment group than in the LVFX treatment group. The carbon chain of metabolites in the OF treatment group is longer than that in the LVFX treatment group, which is the opposite for the PC component. The volcano plot presents the differentially expressed metabolites between the CK and LVFX treatment groups from a statistical perspective. Compared to the OF treatment group, LVFX significantly up-regulated PE-O 16:0–22:4, PE 16:0–14:0, TAG 45:0–FA16:0, PE-P 18:0–16:0, and PE 16:0–16:0; while LVFX significantly down-regulated PC 16:0–22:4 + AcO, PE 18:1–18:3, PC 16:1–18:1 + AcO, and PC 18:1–18:3 +

AcO. Considering that the difference in the contents of OF and LVFX is dextrofloxacin, the above eight differentially expressed metabolites may be caused by dextrofloxacin or by the co-exposure of LVFX and dextrofloxacin. Correlation analysis on the above eight differentially expressed metabolites (as shown in Fig. S8) showed that PC 18:1–18:3 + AcO has the highest correlation with other lipid metabolites. It can be speculated that the stereoselective biological effects of OF at the lipid metabolism level on *R. nigromaculata* are due to differences in the levels of PE-O 16:0–22:4, PE 16:0–14:0, TAG 45:0–FA16:0, PE-P 18:0–16:0, PE 16:0–16:0, PC 16:0–22:4 + AcO, PE 18:1–18:3, PC 16:1–18:1 + AcO, and PC 18:1–18:3 + AcO, with PC 18:1–18:3 + AcO playing a key role.

In a report on the impacts of MC-LR exposure on differentially expressed metabolites of *Pelophylax nigromaculatus* (Zhang et al., 2024), the largest class was lipids and lipid-like molecules (40.07%); glycerophospholipids accounted for the largest proportion, which is consistent with the results of the present study. Glycerophospholipids are important components of biofilms, which can participate in the regulation of fat metabolism, increase the oxidation and consumption of fatty acids, and promote the decomposition and utilization of fat (Hu et al., 2021). Saturated fatty acid (SFA) is the basic lipid component that provides substrates for the generation of fat. Excessive SFA in the liver is associated with liver lipid accumulation and other negative metabolic outcomes (Legrand and Rioux, 2010; Xu et al., 2022). Previous studies have shown that exposure to perfluorooctane sulfonate alters the distribution of fatty acids in frog liver, particularly increasing SFA content and reducing monounsaturated fatty acid and polyunsaturated fatty acid contents, which may contribute to lipid accumulation in the liver (Lin et al., 2022). In this study, compared with the control, exposure treatment increased the SFA content, which may lead to lipid accumulation in the exposed liver. The different effects of OF and LVFX on unsaturated bonds and the carbon chain length of PE and PC components can be attributed to the stereoselective effect of LVFX and dextrofloxacin. Previous studies have shown that OF induces the biosynthesis of unsaturated fatty acids in *Escherichia coli*; a decrease in stearic acid levels and an increase in palmitic acid levels have been reported (KoÇak and Özkul KoÇak, 2020). As is well known, lipid composition is a very important parameter in antibiotic resistance. Fatty acids are the main components of phospholipids and membrane structures and are essential for cellular metabolism. Recent research suggests that fatty acids may be a potential target for antibiotics (Wright and Reynolds, 2007; Parsons and Rock, 2011). Li et al. (2018) analyzed the metabolome structure of *E. coli* under ciprofloxacin stress, showing that ciprofloxacin increased the fatty acid composition in cells and decreased the level of stearic acid. In summary, the observed changes in fatty acid composition may play an important role in the stress adaptation process induced by ofloxacin.

3.6 Levels of proteins related to lipid metabolism

The levels of proteins related to lipid metabolism are shown in Fig. 5. Compared with the control, the level of FAS increased after exposure to LVFX and OF, which may be due to the following reasons. First, LVFX and OF may directly act on FAS, enhance its activity, and increase FAS. Second, LVFX and OF may affect the absorption, decomposition, and synthesis of fatty acids by regulating the activities of proteins related to fatty acid metabolism.

The concentration of ACC was higher in the control group than in the OF treatment group, while it was lower in the LVFX treatment group than in the OF treatment group. Therefore, OF leads to a decrease in the ACC content, and LVFX has a stronger influence. ACC is the key protein of fat metabolism in organisms (Ruderman and Flier, 2001). As a quinolone antibiotic, OF may lead to a decrease in the ability to synthesize biotin (the coenzyme of ACC), which in turn leads to a decrease in the content of ACC, thus affecting fat synthesis (Yu et al., 2021).

The concentration of HSL in the control group was higher than in the OF treatment group, and that in the LVFX treatment group was higher than in the OF treatment group. Therefore, exposure treatment leads to a decrease in HSL content, and the influence of OF is stronger. HSL plays a decisive role in fat mobilization, and it is the rate-limiting protein of fat decomposition. Its activated form is phosphorylated, and can directly act on fat to hydrolyze triglycerides into diglycerides (Grabner et al., 2021). Exposure disrupts the hormonal balance and reduces the HSL content.

Regarding the level of LPL, no significant difference was found between the control group and the LVFX treatment group, but both showed a significant difference compared with the OF treatment group. Therefore, exposure to OF increased the LPL content, on which LVFX had little effect, and dextrofloxacin played a major role. LPL mainly exists in the liver, and if the liver is damaged, this may lead to an increase of LPL content in the liver (Hao et al., 2016). Therefore, exposure to OF may lead to liver damage in tadpoles, thus increasing the LPL content, which is consistent

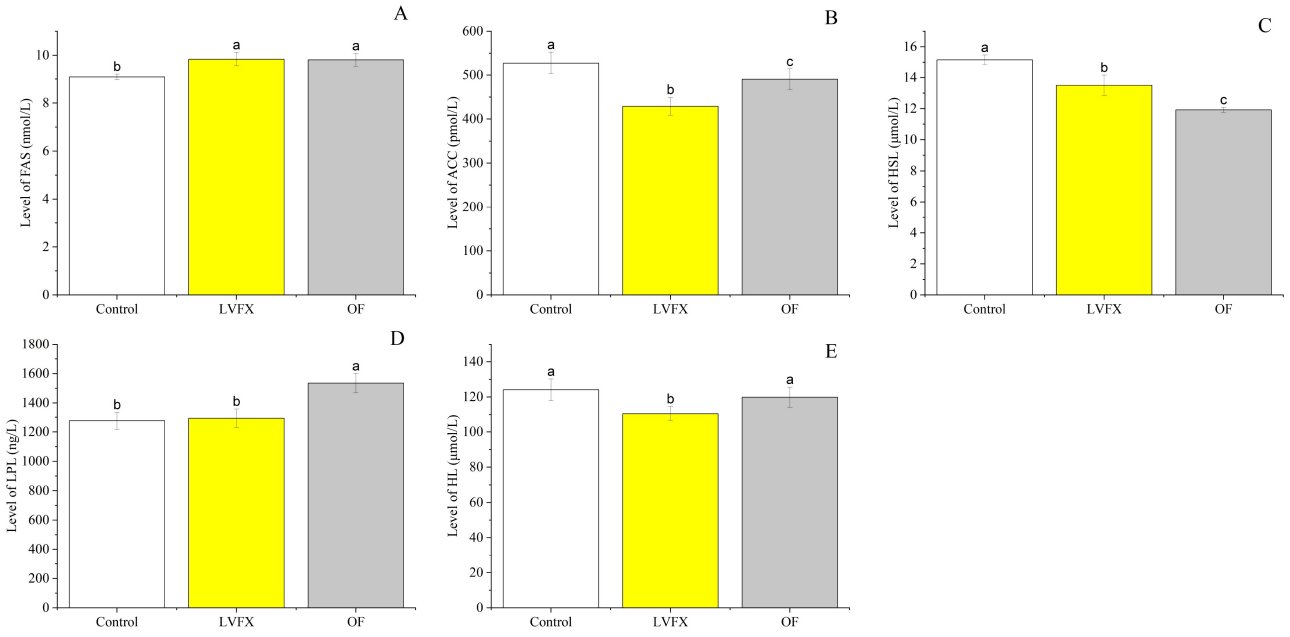

Fig. 5 Levels of fatty acid synthase (FAS) (A), acetyl CoA carboxylase (ACC) (B), hormone sensitive triglyceride lipase (HSL) (C), lipoprotein lipase (LPL) (D) and hepatic lipase (HL) (E) in tadpoles after 75-d exposure. abc: different letters represent statistically significant differences among treatments (SNK) at $P < 0.05$. Error bars indicate the standard deviation.

with the results of liver histological analysis.

Regarding the level of HL, no significant difference was observed between the control group and the OF treatment group, but both differed significantly from the LVFX treatment group. Therefore, LVFX can reduce the content of HL. As a protein related to the endogenous triglyceride metabolism in blood circulation (Khetarpal et al., 2021), HL is synthesized by liver parenchyma cells, saccharified in the endoplasmic reticulum, and then transferred to the Golgi apparatus. In the Golgi apparatus, a mature protein with catalytic activity is formed and transported to the surface of sinus endothelial cells (Kobayashi et al., 2015). LVFX may damage liver function, which may lead to a decrease in HL synthesis in the liver and affect lipid metabolism, which is consistent with the results of liver histological analysis.

In summary, both OF and LVFX promote fat accumulation by stimulating proteins that promote lipogenesis or by inhibiting proteins that promote lipolysis. In other words, both OF and LVFX showed the potential to promote obesity. This result supports the obesity-causing effect of antibiotics (Sang et al., 2021). In addition, the stereoselective biological effect of OF and LVFX on *R. nigromaculata* at the lipid metabolism level is caused by the differential expression of proteins related to lipid metabolism. LVFX promoted FAS but inhibited ACC, HSL, and HL. OF promoted LPL and FAS, but inhibited ACC and HSL. Considering that the difference in content between OF and LVFX is dextrofloxacin, the differential expression of the above lipid metabolism-related proteins is caused by dextrofloxacin, or it may be caused by co-exposure of LVFX and dextrofloxacin. Therefore, it is necessary to further explore the stereoselective binding of LVFX and dextrofloxacin with the above proteins related to lipid metabolism.

3.7 Molecular docking of enzymes related to lipid metabolism with enantiomers

Considering that the contents of ACC, HSL, LPL, and HL reflect enantiomeric selectivity, the binding energies of these four lipid metabolism-related enzymes with ofloxacin enantiomers were further analyzed (Table S2). In general, a docking energy value of less than −7.0 kcal/mol indicates strong binding ability (Yao et al., 2013). Thus, the four lipid metabolism-related enzymes have strong binding ability with ofloxacin enantiomers. The difference in binding energy to ACC between two enantiomers is 1.1, that to HSL between two enantiomers is 1.2, to LPL between two enantiomers is 1.1, and to HL between two enantiomers is 0.9. The binding patterns of lipid metabolism-related enzymes and enantiomers with a difference greater than 1 were further analyzed, and the results are shown in Fig. 6.

For ACC, LVFX forms hydrogen bond interaction

with Val-1981, Leu-2294, and Ser-2301, hydrophobic interaction with GLU-2297, and a halogen bond with TYR-2175. Dextrofloxacin forms hydrogen bond interactions with GLU-1537 and ASN-1541, hydrophobic interaction with Glu-1537, and salt bridge interaction with ARG-1185.

For HSL, LVFX forms hydrogen bond interactions with ASN-314, Val-317, and Glu-420, hydrophobic interactions with ILE-313 and ASN-314, and π-cation interaction with ARG-461. Dextrofloxacin forms hydrogen bond interactions with ASN-314, Val-317, and Glu-420, hydrophobic interactions with ILE-313 and ASN-314, and π-cation interaction with ARG-461.

For LPL, LVFX forms hydrogen bond interactions with TRP-90, Tyr-166, Ser-167, and His-276, hydrophobic interactions with Trp-90, Tyr-129, and ILE-229, π-stacking interaction with Trp-90, and salt bridge interaction with LYS-273. Dextrofloxacin forms hydrogen bond interactions with TRP-90, Tyr-166, Ser-167, and His-276, hydrophobic interactions with Tyr-129 and ile-229, π-stacking interaction with Trp-90, and salt bridge interaction with LYS-273.

In summary, compared with dextrofloxacin, LVFX has stronger binding ability with ACC, HSL, LPL, and HL. Therefore, compared with the control and OF treatment groups, ACC and HL were significantly down-regulated in the LVFX treatment group. However, for HSL and LPL, the effect of OF exceeds that of LVFX. Considering that OF contains equal parts of LVFX and dextrofloxacin, it can be speculated that

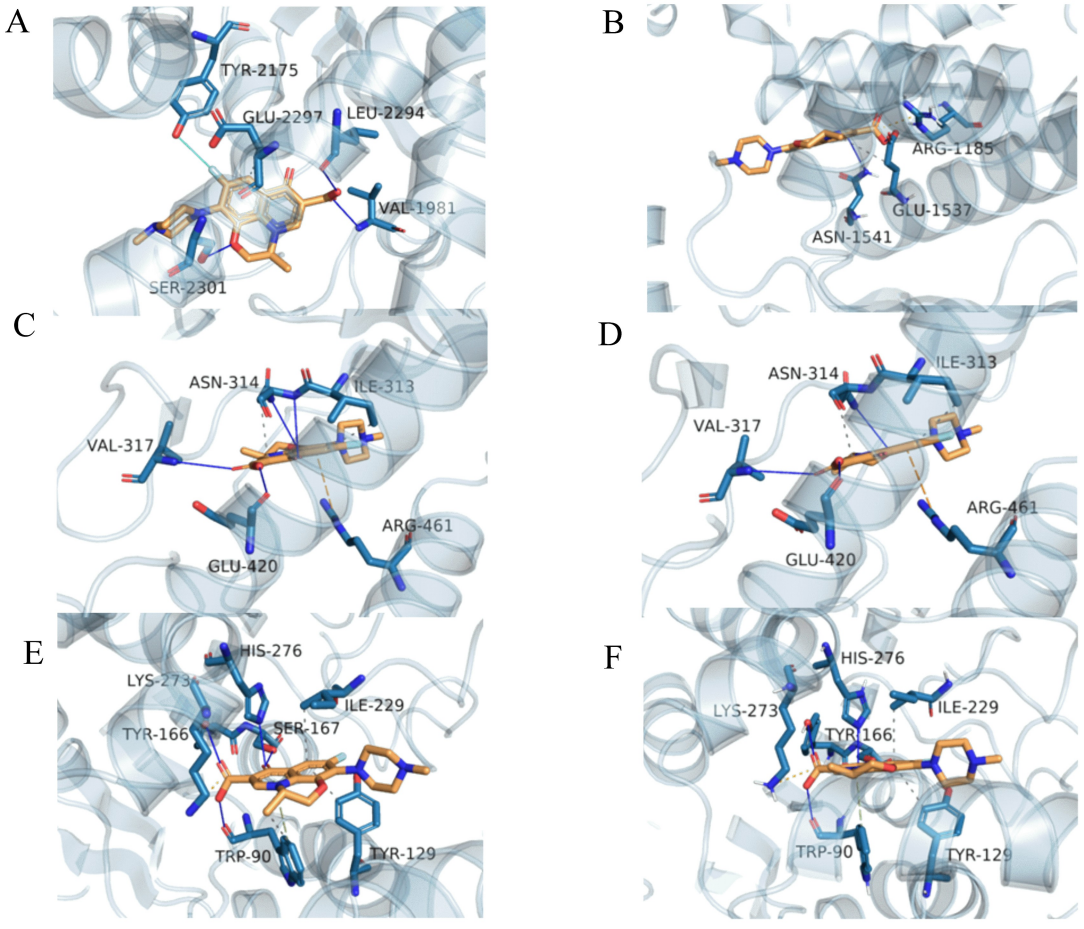

Fig. 6 Molecular docking of proteins with enantiomers of ofloxacin. (A) Molecular docking of LVFX to ACC; (B) Molecular docking of dextrofloxacin to ACC; (C) Molecular docking of LVFX to HSL; (D) Molecular docking of dextrofloxacin to HSL; (E) Molecular docking of LVFX to LPL; (F) Molecular docking of dextrofloxacin to LPL. In the above figure, the orange stick structure represents a small molecule, the cyan cartoon structure is a protein, the blue solid line represents hydrogen bond interaction, the gray dotted line represents hydrophobic interaction, the light yellow dotted line represents salt bridge interaction, the green dotted line represents π-stacking interaction (parallel), the light green dotted line represents π-stacking interaction (perpendicular), the light orange dotted line represents π-cation interaction, and the navy blue line shows halogen bond interaction.

co-exposure of LVFX and dextrofloxacin leads to a stronger effect of OF treatment.

Compared with dextrofloxacin, LVFX treatment has a stronger effect on the inhibition of bodyweight, body length, and developmental stage as well as pathological damage of liver. Therefore, OF exerts a stereoselective inhibitory effect on the growth and development of *R. nigromaculata*, which is consistent with the results at the systemic metabolism level. The effect of OF treatment on *R. nigromaculata* is weaker than that of LVFX treatment. Transcriptome analysis showed that the differentially expressed genes between OF and LVFX were mainly related to immune function. Considering the close relationship between lipid metabolism and immune function, it can be inferred that the difference between OF and LVFX in immune-related pathways is caused by the different effects of OF and LVFX on lipid metabolism. Targeted metabolomics showed that the stereoselective biological effect of OF on *R. nigromaculata* was caused by differences in contents of PE-O 16:0–22:4, PE 16:0–14:0, TAG 45:0–FA16:0, PE-P 18:0–16:0, PE 16:0–16:0, PC 16:0–22:4 + AcO, PE 18:1–18:3, PC 16:1–18:1 + AcO, and PC 18:1–18:3 + AcO in LVFX and dextrofloxacin exposure; especially PC 18:1–18:3 + AcO played a key role. The stereoselective biological effect of OF on *R. nigromaculata* at the lipid metabolism level is caused by the differential expression of enzymes related to the lipid metabolism. Compared with dextrofloxacin, LVFX has a stronger binding ability with ACC, HSL, LPL, and HL. Therefore, compared with the control and OF treatment groups, ACC and HL in LVFX are significantly down-regulated. However, for HSL and LPL, the effect of OF is stronger compared with LVFX. It can be speculated that the co-exposure of LVFX and dextrofloxacin strengthens the effect of OF treatment.

In addition, the results of this study further support the obesity-causing effect of antibiotics. On the one hand, compared with the control, exposure treatment increased the content of saturated fatty acids, which may promote lipid accumulation in the liver upon exposure. On the other hand, OF and LVFX tend to accumulate fat by stimulating enzymes that promote lipogenesis or by inhibiting enzymes that promote lipolysis. In other words, OF and LVFX have the potential to promote obesity.

4 Conclusions

Compared with dextrofloxacin, LVFX treatment has a stronger effect on the inhibition of bodyweight, body length, developmental stage, and pathological damage of liver tissue. Therefore, OF exerts a stereoselective inhibitory effect on the growth and development of *R. nigromaculata*, which is consistent with the results at the systemic metabolism level. Transcriptome analysis showed that the differentially expressed genes between OF and LVFX were mainly immune related. Targeted metabolomics showed that the stereoselective biological effect of OF on *R. nigromaculata* was caused by differences in contents of PE-O 16:0–22:4, PE 16:0–14:0, TAG 45:0–FA16:0, PE-P 18:0–16:0, PE 16:0–16:0, PC 16:0–22:4 + AcO, PE 18:1–18:3, PC 16:1–18:1 + AcO, and PC 18:1–18:3 + AcO. Moreover, two enantiomers of OF were selectively bound to enzymes related to lipid metabolism. This study provides both theoretical and practical references for the accurate evaluation and scientific control of the ecological risk posed by chiral antibiotics.

Conflict of Interests We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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