



Acute Toxicity of Ciprofloxacin, Norfloxacin and Florfenicol to *Misgurnus anguillicaudatus*

Zhen Wang¹, Jinqing Wang^{1*}, Yan Chen¹, Xiaorui Yu¹, Xiaohui Ni¹, Jincheng Wang², Haiyan Sun¹, Yuzhuo Dai¹, Zeyu Li¹, Zhanyu Wang¹

¹Biology and Oceanography College, Weifang University, Weifang, China

²Department of Automotive Engineering, Weifang Vocational College, Weifang, China

Email: *jinqwang@163.com

How to cite this paper: Wang, Z., Wang, J.Q., Chen, Y., Yu, X.R., Ni, X.H., Wang, J.C., Sun, H.Y., Dai, Y.Z., Li, Z.Y. and Wang, Z.Y. (2025) Acute Toxicity of Ciprofloxacin, Norfloxacin and Florfenicol to *Misgurnus anguillicaudatus*. *Open Access Library Journal*, 12: e12850.

<https://doi.org/10.4236/oalib.1112850>

Received: December 12, 2024

Accepted: January 27, 2025

Published: January 30, 2025

Copyright © 2025 by author(s) and Open Access Library Inc.

This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

This study was conducted to investigate the acute toxic effects of ciprofloxacin, norfloxacin and florfenicol, which are widely used antibacterial drugs, on loach. Through a well-planned experimental design, we systematically monitored the physiological and behavioral characteristics of loach under different drug concentrations and exposure time, and evaluated the toxic effects of these drugs. Under specific test conditions, *i.e.* water temperature is maintained at $(10.0 \pm 2.0)^\circ\text{C}$, and the pH value was controlled in the range of 4.6 to 5.7. We tested the acute toxicity of the three drugs on loach with body length of (3.1 ± 0.2) cm and body weight of (1.8 ± 0.1) G. The results showed that the LC50 of ciprofloxacin hydrochloride, norfloxacin and florfenicol to *Misgurnus anguillicaudatus* were 169 mg/L, 276 mg/L and 452 mg/L in 24 hours, and 153 mg/L, 271 mg/L and 447 mg/L in 48 hours, respectively; The median lethal concentration at 72 hours and 96 hours also showed a corresponding trend. At the same time, we calculated that the safe concentrations of the three drugs to loach were 85 mg/L, 78 mg/L and 131 mg/L, respectively. By comprehensive comparison, the order of toxicity intensity of the three drugs to loach was as follows: ciprofloxacin hydrochloride > norfloxacin > florfenicol. This study not only provides a solid scientific basis for the rational use of drugs in aquaculture, but also contributes valuable data resources to the research in related fields, which has important academic value and practical significance.

Subject Areas

Aquaculture, Fisheries & Fish Science, Biochemistry, Bioengineering, Biotechnology

Keywords

Misgurnus Anguillicaudatus, Ciprofloxacin Hydrochloride, Norfloxacin,

1. Introduction

The loach (*Misgurnus anguillicaudatus*), belonging to the family *Misgurnus anguillicaudatus* and the genus [1], is distributed in natural freshwater bodies such as rivers, ditches, paddy fields, ponds, lakes, and reservoirs across China except for the Qinghai-Tibet Plateau and the Tibetan forest areas. It can be said that its population is quite substantial. Generally, loaches live in groups and are small freshwater fish capable of surviving in cold weather conditions. They are oviparous fish [2] [3]. Under natural conditions, they usually start breeding in early April, with their peak spawning period occurring from May to June. They can reproduce at water temperatures ranging from 18°C - 30°C, and they thrive in water temperatures of 22°C - 28°C [4]. The flesh of this fish is delicious, as recorded in “Dian Nan Ben Cao”: it treats sores and dermatitis, promotes blood circulation, and greatly nourishes the yin fluids [5]. It can generally be used in cooking and can also be used for soaking in wine as a tonic.

The tolerance of loaches is very strong and their vitality is extremely tenacious, allowing them to survive under conditions such as low oxygen, ammonia nitrogen, and nitrite nitrogen, making them an important model animal for studying water environmental quality [6]. Moreover, they have a pronounced burrowing habit, which plays a significant ecological role in response to changes in nitrogen and phosphorus nutrient disturbances in paddy fields. This behavior of loaches can significantly influence the nutrient cycling at the water-soil interface in rice fields, affect the vertical distribution of sediment particles, and increase the levels of inorganic nitrogen and total nitrogen in paddy fields [7]-[9].

In this era of rapid technological development, the artificial breeding technology of loaches has become quite mature. Due to their reasonable price and ease of cultivation, loaches are widely welcomed by aquaculturists. However, this fish is also prone to diseases. For common fish diseases, there are already a number of preventive and therapeutic drugs available domestically. However, the permissible dosages for common farmed fish, such as ciprofloxacin hydrochloride, flubendacoumarin, and norfloxacin, may not necessarily be suitable for loaches. In production, many farmers lack a clear understanding of the conditions of their animals or rely on hearsay methods, leading to blind medication use which often fails to achieve the desired therapeutic effects, causing waste and even delaying treatment or worsening the condition, with some cases resulting in poisoning [10]. Therefore, this experiment uses the above three drugs (ciprofloxacin hydrochloride, flubendacoumarin, norfloxacin) to conduct acute toxicity tests on loaches, determining the acute toxicity responses of loaches to ciprofloxacin hydrochloride, norfloxacin, and flubendacoumarin at specific concentrations. The behavior changes, physiological responses, and mortality rates of loaches

exposed to these drugs are observed and recorded. By comparing the toxic effects of different drugs, scientific evidence is provided for rational drug use in aquaculture. Through these drug-induced semi-lethal concentrations (LC50) and safe concentrations (safe concentration) tests, the appropriate drugs for loach farming are determined. It also provides room for research results to provide reference and guidance for the breeding, disease control and treatment of ornamental fish such as guppies, as well as for the healthy and sustainable use of aquaculture water.

2. Materials and Methods

2.1. Experimental Fish

The fish used in the experiment was loach, which was purchased from Shijia Market in Weifang City. The specifications were neat, the response was sensitive, and the fish was healthy. The average total length was 2.9 cm and the average body weight was 1.71 G. The experimental drugs were norfloxacin (Ouyi) GYZZ H13022807 and CSPC Ouyi Pharmaceutical Co., Ltd., with the specification of 0.1g. Ciprofloxacin Hydrochloride (Jingxin) GYZZ H33020388, Zhejiang Jingxin Pharmaceutical Co., Ltd., 0.25 G. 30% Florfenicol Soluble Powder (Huazhu) GYZZ: Veterinary Medicine 120102861, Anhui Keer Pharmaceutical Co., Ltd., Specification 30%.

2.2. Method

The acute toxicity of ciprofloxacin hydrochloride, norfloxacin and florfenicol to *Misgurnus anguillicaudatus* was studied by using biological toxicity test method at room temperature, and six mass concentration gradients were set. The concentrations of florfenicol 30%, ciprofloxacin hydrochloride and norfloxacin were 0, 75, 150, 225, 300, 375 mg/L, 0, 50, 100, 150, 200, 250 mg/L and 0, 170, 200, 280, 260, 290 mg/L respectively. The death number of experimental fish and the state of survival loach were recorded in 24 h, 48 h, 72 h and 96 h, and the water quality was detected every day.

75, 150, 225, 300 and 375 mg/L of ciprofloxacin hydrochloride are respectively dissolved in 100 mL of standby water, and 30 volume percent acetic acid is tentatively dripped into the 100 mL of standby water; firstly, 75 mg of ciprofloxacin hydrochloride is dissolved in 100 ml of water, and 30 volume percent acetic acid is dripped into the 100 ml water, and a glass rod is used for continuously stirring at the same time; Until the ciprofloxacin hydrochloride is completely dissolved in the acetic acid, no sediment, no suspended particles, the dressing change process should be light and slow, try not to disturb the mood of the fish, and the dressing change process should be controlled within 10 minutes. Norfloxacin and florfenicol solutions were prepared in the same manner as ciprofloxacin hydrochloride.

The concentration gradient of the three drugs was determined by the results of the preliminary experiment. Six concentrations of the three drugs were set up, including 0, 75, 150, 225, 300, 375 mg/L of florfenicol 30% and 0, 50, 0.1, 150, 200,

250 mg/L, and norfloxacin concentration of 0, 170, 200, 280, 260, 290 mg/L. The pH, dissolved oxygen (DO), atmospheric pressure (ATM), resistance ($\mu\text{s}/\text{cm}$), TDS, water temperature ($^{\circ}\text{C}$), ORP were measured every day. The experimental water is fully aerated. The experiment took 96 h as an experimental period, and recorded the death number of the experimental fish at 24 h, 48 h, 72 h and 96 h, and measured 9 physical and chemical indexes of the water body.

2.3. Data Collection and Analysis

Total length measured from the tip of the snout to the tip of the caudal fin.

Body length measurement: length from tip of snout to base of caudal fin.

Body height measurement: The vertical height of the highest point of the torso.

Head length measurement: the length from the tip of the snout to the posterior margin of the operculum (excluding the gill membrane).

Excel 2003 software was used for data processing, and SPSS13.0 statistical software was used for one-way analysis of variance. The significant level was $P < 0.05$. When the symbols were different, the difference was significant; when the symbols were the same, the difference was not significant.

3. Results

In order to understand the acute toxicity of ciprofloxacin hydrochloride, norfloxacin and florfenicol to *Misgurnus anguillicaudatus*, the acute toxicity test of three drugs to *Misgurnus anguillicaudatus* with total length of (3.1 ± 0.2) cm and body mass of (1.8 ± 0.1) G was carried out under the conditions of water temperature of $(10.0 \pm 2.0)^{\circ}\text{C}$ and pH of 4.6 - 5.0. The Results showed that the LC50 of ciprofloxacin hydrochloride, norfloxacin and florfenicol to *Misgurnus anguillicaudatus* in 24 h were 169, 276 and 452 mg/L, the LC50 in 48 h were 153, 271 and 447 mg/L, the LC50 in 72 h were 119, 281 and 461 mg/L, respectively. The 96 h median lethal concentrations were 85, 267 and 441 mg/L, respectively. The safe concentrations were 85 mg/L, 78 mg/L and 131 mg/L respectively. The toxicity order of the three drugs to loach was ciprofloxacin hydrochloride > norfloxacin > florfenicol.

Table 1. Estimated norfloxacin parameters.

Norfloxacin	PROBIT(P)	χ^2	P	LC50 (<i>i.e.</i> The 95% confidence Prob = 0.5)	interval I
24(h)	29.176 + 52.223X	1.384	0.709	0.276	0.267~0.285
48(h)	29.484 + 52.066X	4.045	0.257	0.271	0.263~0.281
72(h)	3.188 + 5.789X	34.186	0.000	0.281	0.273~0.291
96(h)	3.068 + 5.348X	28.484	0.000	0.267	0.259~0.277

As can be seen from **Table 1**, The probit model equation of norfloxacin for 24 hours was PROBIT (P) = 29.176 + 52.223X, and the Pearson model goodness of fit test $\chi^2 = 1.384$, P = 0.709, which indicated that the model fit was good. From

this, it can be found that LC50 = 0.276 (*i.e.* Prob = 0.5), and the 95% confidence interval is (0.267 - 0.285).

The probit model equation of norfloxacin at 48 h was PROBIT (P) = 29.484 + 52.066 X, and the Pearson model goodness of fit test $\chi^2 = 4.045$, P = 0.257, which indicated that the model fit was good. From this, it can be found that LC50 = 0.271 (*i.e.* Prob = 0.5), and the 95% confidence interval is (0.263 - 0.281).

The probit model equation of norfloxacin for 72 h was PROBIT (P) = 3.188 + 5.789X, and the Pearson goodness-of-fit test $\chi^2 = 34.186$, P = 0.000, indicating a good model fit. From this, it can be found that LC50 = 0.281 (*i.e.* Prob = 0.50), and the 95% confidence interval is (0.273 - 0.291).

The probit model equation of norfloxacin for 96 h was PROBIT (P) = 3.068 + 5.348X, and the Pearson model goodness of fit test $\chi^2 = 28.484$, P = 0.000, which indicated that the model fit was good. From this, it can be found that LC50 = 0.267 (*i.e.* Prob = 0.50), and the 95% confidence interval is (0.259 - 0.277).

$$SC = 48 \text{ h LC50} \times 0.3 / (24 \text{ h LC50} \div 48 \text{ h LC50})^2$$

The safe concentration is 0.078 G/L, $SC = 0.271 \times 0.3 / (0.276 \div 0.271)^2$

Table 2. Estimated ciprofloxacin parameters.

Ciprofloxacin	PROBIT(P)	χ^2	P	LC50 (<i>i.e.</i> Prob = 0.5)	The 95% confidence interval I
24 (h)	17.158 + 22.255X	1.263	0.738	0.169	0.157 - 0.185
48 (h)	12.191 + 14.934X	0.861	0.835	0.153	0.135 - 0.167
72 (h)	4.420 + 4.785X	12.526	0.006	0.119	0.101 - 0.133
96 (h)	3.525 + 3.294X	8.181	0.04	0.085	0.069 - 0.098

As can be seen from **Table 2**, the probit model equation of ciprofloxacin for 24 hours was PROBIT (P) = 17.158 + 22.255X, and the Pearson model goodness of fit test $\chi^2 = 1.263$, P = 0.738, which indicated that the model fit was good. From this, it can be found that LC50 = 0.169 (*i.e.* Prob = 0.50), and the 95% confidence interval is (0.157 - 0.185).

The probit model equation of ciprofloxacin 48 h was PROBIT (P) = 12.191 + 14.934X, and the Pearson model goodness of fit test $\chi^2 = 0.861$, P = 0.835, which indicated that the model fit was good. LC50 = 0.153 (*i.e.*, when Prob = 0.50), and the 95% confidence interval is (0.135 - 0.167).

The probit model equation of ciprofloxacin for 72 h was PROBIT (P) = 4.420 + 4.785X, and the goodness-of-fit test of Pearson model was $\chi^2 = 12.526$, P = 0.006, which indicated that the model fitted well. From this, it can be found that LC50 = 0.119 (*i.e.* Prob = 0.50), and the 95% confidence interval is (0.101 - 0.133).

The probit model equation of ciprofloxacin 96 h was PROBIT (P) = 3.525 + 3.294X, and the Pearson goodness-of-fit test $\chi^2 = 8.181$, P = 0.04, which indicated that the model fit was good. From this, it can be found that LC50 = 0.085 (*i.e.* Prob = 0.50), and the 95% confidence interval is (0.069 - 0.098).

$$SC = 48 \text{ h LC50} \times 0.3 / (24 \text{ h LC50} \div 48 \text{ h LC50})^2$$

The safe concentration is 0.038 g/L, $SC = 0.153 \times 0.3 / (0.169 \div 0.0153)^2$

Table 3. Estimated values of florfenicol parameters.

Florfenicol	PROBIT(P)	χ^2	P	LC50 (<i>i.e.</i> Prob = 0.5)	The 95% confidence interval I
24 (h)	2.353 + 6.822X	0.003	1.000	0.452	0.443 - 0.462

As can be seen from **Table 3**, The probit model equation of florfenicol for 24 hours was PROBIT (P) = 2.353 + 6.822X, and the Pearson model goodness of fit test $\chi^2 = 0.003$, P = 1.000, which indicated that the model fit was good. From this, it can be found that LC50 = 0.452 (*i.e.* Prob = 0.50), and the 95% confidence interval is (0.443 - 0.462).

$$SC = 48 \text{ h LC50} \times 0.3 / (24 \text{ h LC50} \div 48 \text{ h LC50})^2$$

The safe concentration is 0.131 g/L, $SC = 0.447 \times 0.3 / (0.452 \div 0.447)^2$

Table 4. Changes of daily water quality parameters of ciprofloxacin, florfenicol and norfloxacin solutions in 24 hours.

Drug	Treatment concentration	Indicators					
		pH	ORP (mV)	DO (ppm)	Conductivity ($\mu\text{s}/\text{cm}$)	TDS (ppm)	PSU
Ciprofloxacin	0 g/L	5.33 ± 0.006	240.2 ± 0.058	5.63 ± 0.007	147 ± 0.577	74 ± 0.577	0.07 ± 0.006
	0.05 g/L	4.26 ± 0.006	295.57 ± 0.08	6.85 ± 0.006	872.6 ± 0.33	437 ± 0.577	0.472 ± 0.003
	0.10 g/L	4.63 ± 0.009	286.4 ± 0.058	4.73 ± 0.006	832.3 ± 0.33	416 ± 0.577	0.42 ± 0.006
	0.15 g/L	4.337 ± 0.13	302.8 ± 2.99	4.26 ± 0.009	815 ± 0.577	406 ± 0.577	0.417 ± 0.009
	0.20 g/L	4.24 ± 0.006	305.5 ± 0.058	5.43 ± 0.006	820 ± 0.577	410 ± 0.577	0.417 ± 0.009
	0.25 g/L	4.17 ± 0.006	308.7 ± 0.088	5.98 ± 0.006	841.8 ± 0.33	421 ± 0.577	0.423 ± 0.003
Florfenicol	0 g/L	5.33 ± 0.006	240.2 ± 0.058	5.66 ± 0.007	147 ± 0.577	74 ± 0.577	0.07 ± 0.006
	0.75 g/L	4.04 ± 0.006	316.1 ± 0.058	7.36 ± 0.006	1546.3 ± 0.333	774 ± 0.577	0.77 ± 0.006
	1.50 g/L	3.96 ± 0.006	314.8 ± 0.033	7.08 ± 0.044	2174 ± 0.577	1086 ± 0.577	1.12 ± 0.006
	2.25 g/L	3.78 ± 0.006	315.3 ± 0.058	6.65 ± 0.015	3164 ± 0.333	1417 ± 0.577	1.470 ± 0.006
	3.00 g/L	3.74 ± 0.006	310.5 ± 0.058	6.84 ± 0.006	3443 ± 0.577	1721 ± 0.882	1.830 ± 0.006
	3.75 g/L	3.820 ± 0.006	303.8 ± 0.058	6.62 ± 0.006	4221 ± 0.577	2110 ± 0.333	2.26 ± 0.006
Norfloxacin	0 g/L	5.33 ± 0.006	240.2 ± 0.058	5.663 ± 0.007	147 ± 0.577	74 ± 0.577	0.07 ± 0.006
	0.17 g/L	4.96 ± 0.006	259.7 ± 0.058	5.94 ± 0.006	812 ± 0.577	407 ± 0.577	0.4 ± 0.006
	0.20 g/L	5.75 ± 0.995	278.8 ± 0.058	4.87 ± 0.006	806 ± 0.577	402 ± 0.577	0.47 ± 0.006
	0.23 g/L	4.63 ± 0.006	282.8 ± 0.067	5.75 ± 0.006	730 ± 0.577	365.3 ± 0.88	0.36 ± 0.006
	0.26 g/L	4.68 ± 0.006	282.87 ± 0.03	5.02 ± 0.006	796 ± 0.577	397.7 ± 0.33	0.39 ± 0.006
	0.29 g/L	4.37 ± 0.006	298.4 ± 0.058	4.98 ± 0.02	814 ± 0.577	407 ± 0.577	0.41 ± 0.006

Note: Data are expressed as mean ± standard.

Table 5. Changes of water quality parameters of ciprofloxacin, florfenicol and norfloxacin solutions in 48 h.

Drug	Treatment concentration	Indicators					
		pH	OR P(mV)	DO (ppm)	Conductivity ($\mu\text{s}/\text{cm}$)	TDS (ppm)	PSU
Ciprofloxacin	0 g/L	6.67 ± 0.006	236.77 ± 0.32	3.1 ± 0.006	146 ± 0.77	74 ± 0.577	0.067 ± 0.003
	0.05 g/L	4.36 ± 0.006	271.74 ± 0.09	8.3 ± 0.006	893.3 ± 0.33	447 ± 0.577	0.43 ± 0.006
	0.10 g/L	4.74 ± 0.006	265.9 ± 0.058	6.6 ± 0.006	852.3 ± 0.33	426.67 ± 0.577	0.42 ± 0.01
	0.15 g/L	4.63 ± 0.006	190.97 ± 0.27	6.2 ± 0.01	846 ± 1	424 ± 0.577	0.417 ± 0.003
	0.20 g/L	4.32 ± 0.003	286 ± 0.058	7.6 ± 0.006	834.7 ± 0.33	417 ± 0.577	0.42 ± 0.006
	0.25 g/L	4.26 ± 0.006	289.5 ± 0.058	7.8 ± 0.006	861 ± 0.577	430 ± 0.577	0.42 ± 0.006
Florfenicol	0 g/L	6.67 ± 0.006	236.77 ± 0.32	3.1 ± 0.006	146 ± 0.577	74 ± 0.577	0.067 ± 0.003
	0.75 g/L	4.18 ± 0.009	217.97 ± 0.09	8.8 ± 0.006	1567 ± 0.577	784 ± 0.577	0.78 ± 0.006
	1.50 g/L	4.11 ± 0.006	309.2 ± 0.058	8.5 ± 0.006	2174 ± 0.577	1086 ± 0.577	1.13 ± 0.006
	2.25 g/L	3.92 ± 0.003	313.2 ± 0.058	8.4 ± 0.003	2420 ± 0.333	1214.6 ± 0.333	1.26 ± 0.003
	3.00 g/L	3.92 ± 0.03	313.8 ± 0.033	8.3 ± 0.006	2318 ± 0.577	1157 ± 0.577	0.91 ± 0.006
	3.75 g/L	3.92 ± 0.006	313.6 ± 0.058	8.4 ± 0.003	2360 ± 0.333	1181 ± 0.577	1.12 ± 0.003
Norfloxacin	0 g/L	6.67 ± 0.006	236.77 ± 0.32	3.1 ± 0.006	146 ± 0.577	74 ± 0.577	0.067 ± 0.003
	0.17 g/L	5.07 ± 0.01	274.8 ± 0.058	6.6 ± 0.006	829 ± 0.577	414 ± 0.577	0.41 ± 0.006
	0.20 g/L	4.82 ± 0.006	293.5 ± 0.058	6.6 ± 0.009	430 ± 0.577	217 ± 0.577	0.21 ± 0.006
	0.23 g/L	4.67 ± 0.003	297.9 ± 0.033	7.4 ± 0.006	392 ± 0.577	197 ± 0.577	0.2 ± 0.006
	0.26 g/L	4.75 ± 0.006	297.7 ± 0.058	7.8 ± 0.003	450 ± 0.577	220 ± 0.577	0.197 ± 0.003
	0.29 g/L	4.32 ± 0.003	313 ± 0.058	7.4 ± 0.009	436 ± 0.577	218 ± 0.577	0.2 ± 0.006

Note: Data are expressed as mean \pm standard.

Table 6. Changes of water quality parameters of ciprofloxacin, florfenicol and norfloxacin solutions in 72 h.

Drug	Treatment concentration	Indicators					
		pH	ORP (mV)	DO (ppm)	Conductivity ($\mu\text{s}/\text{cm}$)	TDS (ppm)	PSU
Ciprofloxacin	0 g/L	5.75 ± 0.006	235.3 ± 0.058	4.64 ± 0.009	177 ± 0.577	89 ± 0.577	0.08 ± 0.006
	0.05 g/L	4.52 ± 0.006	262.8 ± 0.058	9.55 ± 0.006	944 ± 0.577	472 ± 0.577	0.43 ± 0.003
	0.10 g/L	4.81 ± 0.006	258.6 ± 0.058	7.56 ± 0.006	877 ± 0.577	438 ± 0.577	0.43 ± 0.003
	0.15 g/L	4.74 ± 0.006	267.3 ± 0.333	8.33 ± 0.006	870 ± 0.577	433 ± 0.882	0.43 ± 0.006
	0.20 g/L	4.35 ± 0.009	279 ± 0.058	8.47 ± 0.006	848 ± 0.577	424 ± 0.577	0.42 ± 0.003
	0.25 g/L	4.26 ± 0.006	283.4 ± 0.058	8.89 ± 0.006	870 ± 0.577	435 ± 0.577	0.43 ± 0.006
Florfenicol	0 g/L	5.75 ± 0.006	235.3 ± 0.058	4.58 ± 0.009	177 ± 0.577	89 ± 0.577	0.08 ± 0.006
	0.75 g/L	4.24 ± 0.006	291.5 ± 0.058	10.4 ± 0.006	1558 ± 0.577	778 ± 0.333	0.78 ± 0.01
	1.50 g/L	4.15 ± 0.01	292.5 ± 0.033	9.81 ± 0.028	2196.3 ± 0.33	$1098. \pm 0.33$	1.13 ± 0.006

Continued

	2.25 g/L	4.02 ± 0.006	297.3 ± 0.033	9.53 ± 0.006	2825 ± 0.577	1413 ± 0.577	1.47 ± 0.006
	3.00 g/L	3.97 ± 0.006	297.2 ± 0.153	9.48 ± 0.006	3481 ± 0.577	1741 ± 0.577	1.85 ± 0.006
	3.75 g/L	4.03 ± 0.009	295.4 ± 0.058	9.34 ± 0.006	4297 ± 0.577	2149 ± 0.577	2.31 ± 0.006
Norfloxacin	0 g/L	5.75 ± 0.006	235.3 ± 0.058	4.6 ± 0.009	177 ± 0.577	89 ± 0.577	0.08 ± 0.006
	0.17 g/L	4.92 ± 0.006	256.7 ± 0.058	8.33 ± 0.06	954.6 ± 0.333	477 ± 0.577	0.47 ± 0.003
	0.20 g/L	5.02 ± 0.006	273.5 ± 0.058	8.11 ± 0.006	866 ± 0.577	433 ± 0.577	0.43 ± 0.006
	0.23 g/L	4.8 ± 0.01	277.5 ± 0.033	8.24 ± 0.006	791 ± 0.577	397 ± 0.577	0.39 ± 0.003
	0.26 g/L	4.84 ± 0.01	277.5 ± 0.088	8.4 ± 0.006	874 ± 0.577	424 ± 0.577	0.41 ± 0.006
	0.29 g/L	4.59 ± 0.003	294.3 ± 0.173	7.58 ± 0.009	838 ± 0.577	418 ± 0.577	0.41 ± 0.006

Note: Data are expressed as mean ± standard.

Table 7. Changes of water quality parameters of ciprofloxacin, florfenicol and norfloxacin solutions in 96 h.

Drug	Treatment concentration	Indicators					
		pH	ORP (mV)	DO (ppm)	Conductivity (µs/cm)	TDS (ppm)	PSU
Ciprofloxacin	0 g/L	6.34 ± 0.006	229.5 ± 0.058	5.15 ± 0.006	164 ± 0.577	82 ± 0.577	0.08 ± 0.006
	0.05 g/L	4.14 ± 0.006	264.8 ± 0.058	9.48 ± 0.006	951 ± 0.577	476 ± 0.577	0.48 ± 0.006
	0.10 g/L	4.62 ± 0.006	257.8 ± 0.058	7.55 ± 0.009	8944 ± 0.577	446 ± 0.577	0.45 ± 0.006
	0.15 g/L	4.58 ± 0.006	264.8 ± 0.058	7.34 ± 0.006	882 ± 0.577	441 ± 0.577	0.45 ± 0.009
	0.20 g/L	4.23 ± 0.003	278.8 ± 0.173	7.98 ± 0.006	853 ± 0.577	426 ± 0.577	0.42 ± 0.006
	0.25 g/L	4.17 ± 0.006	282.8 ± 0.058	8.38 ± 0.006	874 ± 0.577	588 ± 0.577	0.42 ± 0.006
Florfenicol	0 g/L	6.34 ± 0.006	229.5 ± 0.058	5.15 ± 0.006	164 ± 0.577	82 ± 0.577	0.08 ± 0.006
	0.75 g/L	4.15 ± 0.009	286.8 ± 0.058	9.06 ± 0.003	1561 ± 0.577	781 ± 0.577	0.78 ± 0.006
	1.50 g/L	4.11 ± 0.006	288.6 ± 0.058	9.12 ± 0.006	2202 ± 0.577	1101 ± 0.577	1.13 ± 0.009
	2.25 g/L	3.96 ± 0.006	294.4 ± 0.088	9.15 ± 0.009	2798 ± 0.577	1398 ± 0.577	1.46 ± 0.006
	3.00 g/L	3.92 ± 0.006	293.6 ± 0.058	8.96 ± 0.006	3535 ± 0.577	1767 ± 0.577	1.87 ± 0.009
	3.75 g/L	3.98 ± 0.006	291.7 ± 0.058	9.04 ± 0.006	4338 ± 0.577	2169 ± 0.577	2.32 ± 0.003
Norfloxacin	0 g/L	6.34 ± 0.006	229.5 ± 0.058	5.15 ± 0.006	164 ± 0.577	82 ± 0.577	0.08 ± 0.006
	0.17 g/L	5.25 ± 0.006	255.6 ± 0.058	4.97 ± 0.006	968 ± 0.577	484 ± 0.577	0.477 ± 0.003
	0.20 g/L	4.87 ± 0.006	273.7 ± 0.058	6.22 ± 0.006	867 ± 0.577	433 ± 0.882	0.43 ± 0.006
	0.23 g/L	4.76 ± 0.006	278.6 ± 0.058	6.76 ± 0.009	797 ± 0.577	398 ± 0.577	0.38 ± 0.006
	0.26 g/L	4.84 ± 0.012	277.3 ± 0.088	6.63 ± 0.006	855 ± 0.577	427 ± 0.577	0.43 ± 0.003
	0.29 g/L	4.34 ± 0.009	294.5 ± 0.088	7.66 ± 0.009	843 ± 0.577	422 ± 0.577	0.42 ± 0.009

Note: Data are expressed as mean ± standard.

It can be seen from **Tables 4-7**. The results of water quality analysis showed that there were significant differences in water quality indexes between the drug

treatment group and the control group. With the increase of ciprofloxacin concentration, the pH value decreased from 4.20 to 4.09 on the first day, from 4.35 to 4.25 on the second day, from 4.50 to 4.20 on the third day, and from 4.10 to 4.09 on the fourth day. PH of florfenicol decreased from 4.00 to 3.90 on the first day and from 4.00 to 3.90 on the second day, 20 to 3.95, Day 3 from 4.22 to 4.0, and Day 4 from 4.19 to 3.99. PH of norfloxacin decreased from 4.98 to 4.38 on the first day, from 5.08 to 4.37 on the second day, from 4.95 to 4.58 on the third day, and from 5.25 to 4.37 on the fourth day.

During the whole test period, the pH value of the control group was basically stable, and the fluctuation range was within ± 0.2 units, which indicated the stability of the test water quality.

ORP reflects the relative strength between oxidants and reductants in water, and is a comprehensive index to judge the redox state of water quality. The ORP of loach exposed to different concentrations of ciprofloxacin, florfenicol and norfloxacin for 4 days was as follows: the ORP of ciprofloxacin increased from 296 to 309 on the first day, from 271 to 289 on the second day, and from 263 to 283 on the third day; the fourth day increased from 264 to 284. The ORP of florfenicol decreased from 317 to 304 on the first day, increased from 311 to 313 on the second day, and increased from 291 to 294 on the third day. Day 4 increased from 288 to 291. Norfloxacin ORP increased with increasing concentrations from 260 to 299 on the first day, from 275 to 313 on the second day, from 258 to 293 on the third day, and from 230 to 294 on the fourth day. Compared with the control group, the ORP value was higher, which may indicate that the addition of ciprofloxacin promoted the occurrence of some oxidation reactions in the water body, such as the degradation of ciprofloxacin. It may adversely affect the survival of loach and increase the risk of oxidative stress.

The dissolved oxygen of water quality is an important index to measure the self-purification ability of water body, biological activity and the living conditions of aquatic organisms. Norfloxacin, ciprofloxacin and florfenicol have toxic effects on loach, which may affect their physiological activities, such as respiration, and indirectly affect the dissolved oxygen content in water. The monitoring results showed that the dissolved oxygen of loach in different concentrations of ciprofloxacin, florfenicol and norfloxacin solutions for 4 days changed as follows, with the increase of time and drug concentration: the dissolved oxygen of ciprofloxacin decreased from 6.91 to 6.00 on the first day, from 8.34 to 7.91 on the second day, from 9.54 to 9.00 on the third day; On the fourth day, it dropped from 9.52 to 8.26. The DO of florfenicol decreased from 7.32 to 6.90 on the first day, from 8.88 to 8.25 on the second day, from 10.00 to 9.28 on the third day, and from 9.00 to 9.02 on the fourth day. The DO of norfloxacin decreased from 5.98 to 4.99 on the first day, increased from 6.76 to 7.99 on the second day, decreased from 8.26 to 7.77 on the third day, and decreased from 4.99 to 7.79.

The analysis of water conductivity is also an important aspect of assessing water quality. Conductivity reflects the content of dissolved salts in water. The results

showed that the changes of EC of loach in different concentrations of ciprofloxacin, florfenicol and norfloxacin were as follows: EC of ciprofloxacin decreased from 873 to 841 on the first day, from 894 to 860 on the second day, and from 944 to 870 on the third day; The fourth day dropped from 951 to 873. Florfenicol EC increased with increasing concentration from 1546 to 4220 on day 1; from 1566 to 2360 on day 2; from 1558 to 4297 on day 3; from 1561 to 4338 on day 4; norfloxacin EC increased with increasing concentration from 811 to 813 on day 1; from 829 to 436 on day 2; the third day dropped from 955 to 839; the fourth day dropped from 968 to 842. The conductivity of the treatment group was significantly higher than that of the control group, which may indicate that the addition of norfloxacin, ciprofloxacin and florfenicol led to the increase of dissolved salts in the water. This may be due to degradation products of norfloxacin, ciprofloxacin, florfenicol, reaction products with other substances, or ionization of the antibiotic itself. The increase of conductivity may have adverse effects on the living environment of loach, such as increasing the burden of osmotic pressure regulation.

The results showed that the TDS of loach in different concentrations of ciprofloxacin, florfenicol and norfloxacin solutions for 4 days changed as follows, with the increase of test time and drug concentration: the TDS of ciprofloxacin decreased from 436 to 420 on the first day, from 447 to 430 on the second day, from 472 to 435 on the third day; Day 4 increased from 475 to 588. Florfenicol TDS increased with increasing concentration from 773 to 2110 on day 1; from 783 to 1180 on day 2; from 779 to 2149 on day 3; from 781 to 2169 on day 4; Norfloxacin TDS increased with increasing concentration from 406 to 407 on day 1; from 414 to 218 on day 2; the third day dropped from 477 to 419; the fourth day dropped from 484 to 421.

The results showed that the PSU of *Misgurnus anguillicaudatus* in different concentrations of ciprofloxacin, florfenicol and norfloxacin solutions for 4 days changed as follows, with the increase of test time and drug concentration: PUS decreased from 0.43 to 0.42 on the first day and from 0.44 to 0.43 on the second day with the increase of ciprofloxacin concentration; the third day dropped from 0.47 to 0.43; the fourth day dropped from 0.47 to 0.43. PUS of florfenicol increased from 0.78 to 2.26 on the first day, from 0.79 to 1.12 on the second day, from 0.79 to 2.30 on the third day, from 0.79 to 2.32 on the fourth day, and PUS of norfloxacin increased from 0.4 to 0.41 on the first day; the second day was from 0.41 to 0.21; the third day was from 0.47 to 0.41; the fourth day was from 0.48 down to 0.42.

The changes of water temperature of *Misgurnus anguillicaudatus* in different concentrations of ciprofloxacin, florfenicol and norfloxacin solutions for 4 days were as follows: Ciprofloxacin, florfenicol, and norfloxacin at all concentrations (including controls) were 10.92°C at 24 hours, 9.23°C at 48 hours, 7.85°C at 72 hours, and 7.57°C at 96 hours.

The mortality of *Misgurnus anguillicaudatus* seedlings in different concentrations of ciprofloxacin solution is shown in **Figure 1**. It can be seen from **Figure 1**

that with the extension of the test time and the increase of the drug concentration, the toxicity of ciprofloxacin to the young loach increased, and the mortality of the young loach increased. The young loach swam rapidly in the ciprofloxacin solution of high concentration, lay at the bottom of the barrel after poisoning, ate less, and floated on the surface of the water (not swimming) before death. The belly faces the surface of the water, and some of them lie on their sides on the surface of the water (some of them will move when they are touched with the strainer, and the ones that do not move will recover their vitality when they are taken out of the surface of the water). After death, the body color of the fish is gray, and most of them float on the surface of the water, and some of them sink to the bottom, and a large amount of mucus appears on the body surface. When the concentration of ciprofloxacin was 50 mg/L, the fish sank to the bottom and died in 40 hours, and the mortality was 0% in 24 hours. When the concentration of ciprofloxacin reached 250 and 200 mg/L, the mortality reached 100% in 24 hours. When the concentration reached 150 mg/L, death occurred at 24 H, and the mortality rate reached 60% at 96 H. There was no death in the control group during the experiment.

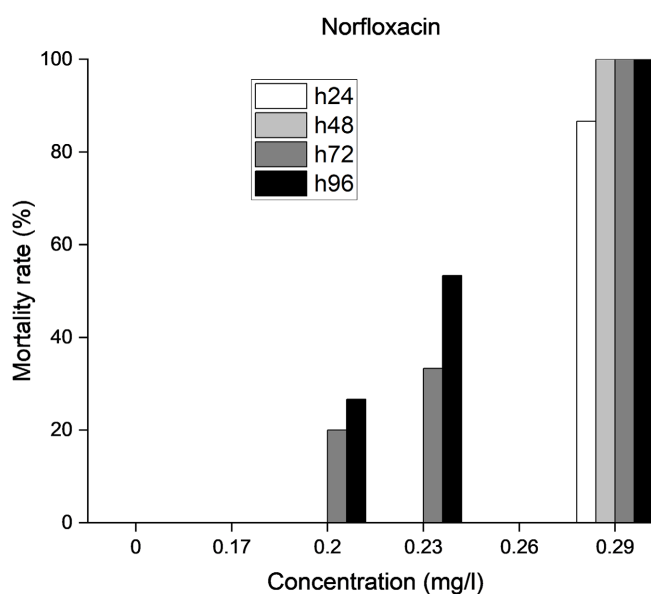


Figure 1. Mortality of loach seedlings at different concentrations of ciprofloxacin.

The mortality of *Misgurnus anguillicaudatus* seedlings in different concentrations of norfloxacin solution is shown in **Figure 2**. It can be seen from **Figure 2** that the mortality of loach in norfloxacin is different with the increase of concentration. When the concentration is 170mg/L, the test fish is not dead. When the concentration was 200 mg/L, the test fish did not die at 24 and 48 hours, but died at 55 hours, and the mortality was 27% at 96 hours. When the concentration was 230 mg/L, no fish died at 24 and 48 hours, but died at 70 hours, and the mortality rate was 53% at 96 hours. When the concentration was 260 mg/L, no fish died. When the concentration was 290 mg/L, the mortality rate reached 87% at 24 H.

There were no deaths in the control group during the trial. The mortality of norfloxacin in this experiment did not increase with the increase of concentration, and it did not die at 260 mg/L. The reason may be related to the size of the fish, the constitution and immunity of the fish during the experiment.

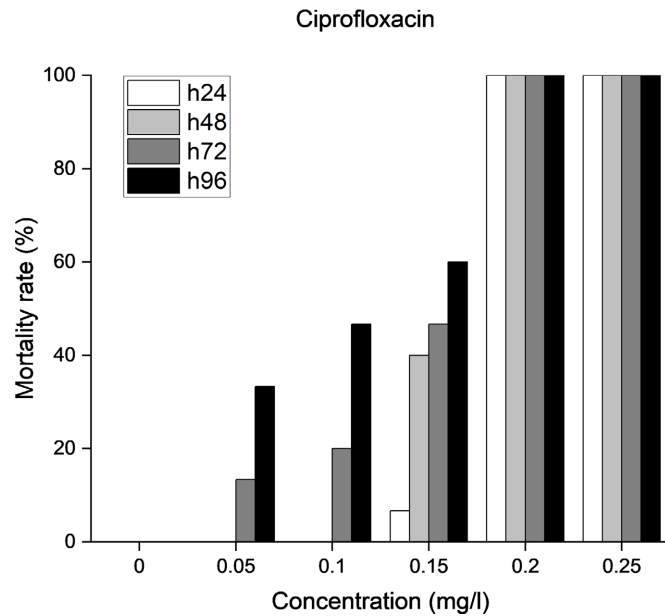


Figure 2. Mortality of loach seedlings in different concentrations of norfloxacin solution.

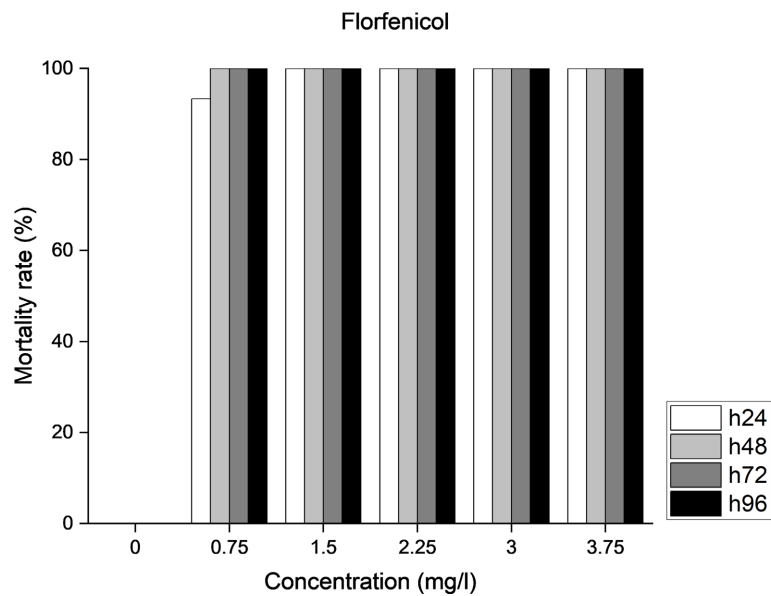


Figure 3. Mortality of loach seedlings in different concentrations of florfenicol solution.

The mortality of loach seedlings in different concentrations of florfenicol solution is shown in **Figure 3**. It can be seen from **Figure 3** that with the extension of the test time and the increase of the drug concentration, the toxicity of florfenicol to juvenile loach increased, and the mortality of loach increased significantly. After the fish was poisoned, it was manic and restless. It swam rapidly in the test

barrel. With the increase of time, it did not respond when it was touched by foreign objects in the liquid medicine. When it was fished out and put into paper towels, it would twist its body and swim up and down when it was put back into the liquid medicine. After death, most of the body will sink to the bottom of the barrel, a few will float on the surface of the water, the body will be straight, and the whole body will be covered with a layer of white membrane (can fall off). When the concentration of florfenicol was 75 mg/L, the experimental fish died in 14 hours, and the mortality rate was 94% in 24 hours. When the concentration of florfenicol was 150, 225, 300, 375 mg/L, the mortality rate of the experimental fish reached 100% in 24 hours, and the control group did not die.

4. Discussion

For the acute toxicity test of loach, there have been studies on florfenicol, norfloxacin, enrofloxacin and other drugs in aquatic animals at home and abroad, and the effects of physiological differences, pharmacology, water quality and other factors on the pharmacokinetics and residue elimination of these drugs in fish were summarized and analyzed. However, there are no significant results in the study of ciprofloxacin hydrochloride, florfenicol and norfloxacin. Therefore, the loach was used as the experimental fish, and ciprofloxacin hydrochloride, florfenicol and norfloxacin were used as the experimental drugs to study their toxicity, aiming at finding the safe range of drug use in fish farming, reducing the harm of drugs to loaches and other similar fish, and promoting the sustainable and healthy development of large-scale loach aquaculture. The evaluation standard of toxic substances to fish shows [11]: acute toxicity 96 h median lethal concentration less than 0.1 mg/L is highly toxic, 0.1 - 1.0 mg/L is highly toxic, 1.0 - 10.0 mg/L is toxic, and higher than 10.0 mg/L is low toxic. The results showed that the median lethal concentrations of ciprofloxacin hydrochloride, norfloxacin and florfenicol to juvenile *Misgurnus anguillicaudatus* were 85, 267 and 441 mg/L, respectively, which belonged to low toxicity drugs.

There is a view that in the study of toxic effects of pollutants in fish, larvae should be used as experimental materials as far as possible, so as to avoid the problem of high toxicity data caused by too large test fish. However, if we simply carry out toxicity testing on fish at a certain growth stage, the experimental data obtained will be too single to obtain systematic toxicity data, nor can we carry out comprehensive toxicity assessment. Therefore, in order to get more comprehensive information about the toxic effects of pollutants on fish and the mechanism of toxicity, it is necessary to carry out exposure experiments on fish at different growth stages, so that the data obtained will be more comprehensive and systematic, and can better provide basic data for the assessment of the toxic effects of pollutants.

In this experiment, the safe concentration of ciprofloxacin was 38 mg/L. In the paper published by Fang Yingchun and other researchers [12], the same drug was used to study the lethal concentration of guppy, in which the safe concentration of ciprofloxacin hydrochloride was 300 mg/L, and the safe concentration of

ciprofloxacin hydrochloride in guppy measured by Fang Yingchun and other researchers was 3.48 times; in this experiment, the 48 h semi-lethal concentration of ciprofloxacin was 153 mg/L, while in Fang Yingchun's experiment, the semi-lethal time was an important index to measure the speed of death caused by toxic substances. In Fang Yingchun's experiment, the 48 h semi-lethal concentration of levofloxacin hydrochloride was 365 mg/L, and the semi-lethal concentration was 2. The average body length of guppies used by Fang Yingchun *et al.* is 0.3 cm longer than that of loaches used in this experiment, but the average body weight is only 1/4 of that of loaches, but the safe concentration and median lethal concentration are quite different, which shows that the same aquatic fish are similar in size, but the drug concentrations used are different. If we only rely on the study of the lethal concentration of a single fish to explain the applicable concentration of the drug in the aquatic products as a whole, this practice is not rigorous. Ciprofloxacin is the main metabolite of enrofloxacin, which is widely used because of its broad antibacterial spectrum, strong bactericidal activity and rapid onset of action.

In this study, the safe concentration of norfloxacin was 78 mg/L. In the study of Fang Yingchun *et al.*, the safe concentration of norfloxacin in guppy was 200 mg/L, which was 2.6 times that of loach. The 48 h median lethal concentration of norfloxacin in this test was 271 mg/L, while the 48 h median lethal concentration of norfloxacin in Fang Yingchun *et al.* was 276 mg/L [12] [13]. Different kinds of fish must refer to the literature and ask professionals before medication, and do not blindly use drugs by themselves.

Florfenicol has strong bacteriostatic effects, fast absorption for loach, and no toxicity and side effects. At present, florfenicol has replaced a variety of antibiotics for the prevention and treatment of animal diseases caused by bacteria [14]. The safe concentration of florfenicol to loach in this experiment is 131 mg/L, which is lower than research of Jian Yuxia [15] and Wang Ruixue *et al.* [16]. Florfenicol will act on the liver and kidney of animals. If too much florfenicol is added, it will cause very serious harm to the body of animals. Therefore, the drug concentration must be controlled when using drugs to avoid unnecessary losses. In the effect of [17] [18] florfenicol on zebrafish, when the concentration of florfenicol is 0.01 - 100 mg/L, florfenicol could affect the hatching rate of zebrafish embryos and destroy the antioxidant system in embryos. Therefore, the concentration of florfenicol should not be too high during the experiment. In the high dose group of florfenicol (40 mg/kg), the liver and kidney cells were damaged in a dose-dependent manner, and the damage was not repaired after 7 days of feeding. It is recommended that the dosage in production should not be higher than 20 mg/kg to avoid tissue damage, reduced utilization and environmental pollution [19].

In the acute toxicity test of four kinds of commonly used fish medicines including Yang Qichao [20] on loach, in the toxicity test of formalin on loach, the dead loach appeared stiff, the fin rays were completely open, the body color was white, the body surface secreted a large amount of mucus and white flocculent attached to the body surface, while the surviving loach had slow reaction and could recover

in clear water. According to the research of ichthyology, there is a kind of mucus cell that can secrete mucus in the body tissue of fish, such as skin, gill and intestinal epithelium of digestive tract, and there are many active substances in the mucus secreted by this cell, such as immunoglobulin and various hydrolytic enzymes. When fish are affected by external factors (such as high temperature, poisons, immunogenic substances, etc.) Under stress, the internal stress response of fish will cause changes in the distribution and number of mucus cells, which will lead to an increase in mucus secretion. In this experiment, the dead loach also showed white body color, a large amount of mucus secretion on the body surface, eye congestion, and no obvious bleeding at the base of fins and gills. There are no poisoning symptoms among the surviving loaches, and if they are affected by external factors (such as fishing with a net), they react quickly. In He Guosen's acute toxicity experiment on loach, the loach in the povidone-iodine experimental group swam rapidly, collided everywhere, and even jumped. After poisoning, the loach slowly lost its balance, sank to the bottom of the water, and its breathing was weakened until it died [21]. The three drugs used in this experiment were relatively quiet and had no obvious symptoms, similar to potassium permanganate, and most of the loaches in the experiment liked to sink to the bottom, and some of them would float on the surface of the water, but the time was short, the swimming was slow, and a few of them died gradually with the passage of time.

To sum up, through this experiment, we can see that ciprofloxacin hydrochloride, florfenicol and norfloxacin still have certain toxicity to loach. As the saying goes: "Drugs are three poisons", once the dosage of drugs is too much, it will cause a large number of deaths, if the dosage is too much in the actual breeding, it will cause irreparable losses, so once again remind the majority of breeders to be careful about the dosage, the test is for reference only. At the same time, appropriate room temperature, water temperature and water quality must be maintained during breeding to prevent loach from reducing production due to environmental problems, and timely observation and attention should be paid to the state of loach during breeding. Through this experiment, we have a certain understanding of the habits of loach, and give corresponding suggestions for the breeding of loach and other fish, hoping that it will be helpful to breeders, and also hope that readers can learn the relevant knowledge of loach breeding by reading this article.

Funding

Weifang Science and Technology Development Plan (2023GX018); Industry-University Cooperation and Collaborative Education Project of the Ministry of Education (220601018015222); in 2022, Shandong Jinshuiwan Koi Fish Breeding Co., Ltd. aquaponics horizontal project; in 2024, Shanghai Yingxi Fruit and Vegetable Professional Cooperative will have a horizontal project of aquaponics koi farming.

Conflicts of Interest

The authors declare no conflicts of interest.

References

- [1] Li, X.T. (2019) Natural Perception Series, Beijing Natural Stories. Commercial Press, 367.
- [2] Li, X., Ma, C., Qin, Y.J., *et al.* (2014) Establishment and Characterization of Fin Cell Lines from Different Ploidy Oriental Weatherfish *Misgurnus anguillicaudatus*. *Acta Hydrobiologica Sinica*, **38**, 150-157.
- [3] Zhao, X. (2012) The Genetic Diversity Analysis of *Paramisgurnus dabryanus* in the Yellow River Basin. Henan Normal University.
- [4] Li, H.G. (2015) Key Technologies for Healthy and Efficient Culture of Loach. Zhongyuan Farmers Publishing House, 3-4.
- [5] Lao, C.H. (2014) Food Therapy and Health. China Traditional Chinese Medicine Press, 259.
- [6] Wang, J.Q., Zhou, W.Z. and Zhang, J.Q., *et al.* (2016) Research Progress on behavior Ecology of Burrowing Loach *Misgurnus anguillicaudatus*. *Acta Agriculturae Shanghai*, No. 2, 144-149.
- [7] Sun, G., Fang, Y., Dong, G., Lu, M., *et al.* (2009) The Effects of *Misgurnus anguillicaudatus* Bioturbation on the Vertical Distribution of Sediment Particles in Paddy Field. *Journal of Anhui Agricultural Sciences*, **37**, 703-704.
- [8] Yang, L.G. (2011) Response of Paddy Soil Nutrients to Bioturbation of *Misgurnus anguillicaudatus*. *Journal of Anhui Agricultural Sciences*, **39**, 3884-3885.
- [9] Sun G., Fang, Y., An Y.H. and Yang, X.Y. (2009) Bioturbation Effect of *Misgurnus anguillicaudatus* on Nitrogen Dynamic in Surface Water of Paddy Field. *Journal of Ecology and Rural Environment*, **25**, 39-43.
- [10] Wu, G.T. (2004) Key Points of Medication for Aquatic Animals. *Fisheries Modernization*, No. 1, 24-29.
- [11] State Environmental Protection Administration (2006) Methods for Monitoring and Analysis of Water and Wastewater. China Environmental Science Press, 777-781.
- [12] Fang, Y.C., Qi, Y., Li, Y., *et al.* (2019) Acute Toxicity Experience to Guppy with Ciprofloxacin HCl, Enrofloxacin and Norfloxacin. *Journal of Shenyang University (Natural Science)*, **24**, 15-17.
- [13] Liang, X.M. (2010) Toxicity of Norfloxacin and Triclosan to *Xiphophorus helleri* (Swordtail Fish). Jinan University.
- [14] Guo, S.Z. and Wu, X.J. (2008) Florfenicol. *Marine and Fishery*, No. 5, 23-24.
- [15] Jian, Y.X., Li, L., Wang, X. L., *et al.* (2003) Acute Toxicity of Four Commonly Used Aquatic Medicine to Juveniles *Nibeia albiflora*. *Fishery Science and Technology Information*, **49**, 143-149.
- [16] Wang, R.X., Wang, D., Li, S.W., *et al.* (2012) Pharmacokinetics and Tissue Distribution of Florfenicol and Florfenicol Amine in *Acipenser baerii*. *Journal of Shanghai Ocean University*, **21**, 568-574.
- [17] He, L.J., Zhang, L. and Li, Y.M., *et al.* (2022) Effects of Florfenicol and Its Metabolite Florfenicol Amine on Oxidative Stress in Zebrafish Embryos. *Quality and Safety of Agricultural Products*, No. 3, 10-16.
- [18] Zhang, Y.G., Wang, Z. and Zhang, K.X., *et al.* (2022) Joint Toxicity of Enrofloxacin and Florfenicol on Soft-Shell Turtle. *Scientific Fish Farming*, No. 12, 49-51.
- [19] Wang, W.L., Luo, L., Jiang, L., *et al.* (2014) Blood Concentration and Histological Toxicology of Different Dosages of Florfenicol in Gift *Nile tilapia*. *Journal of Shanghai Ocean University*, **23**, 90-94.

- [20] Yang, Q.C., Wan, Q., Zhao, J.F., *et al.* (2006) Acute Toxicity Test of Four Commonly Used Fish Drugs on Loach. *Water Resources and Fisheries*, **26**, 93-95.
- [21] He, G.S., Zeng, Z.Z. and Chen, D.H. (2014) Acute Toxicity Test of Four Drugs to *Misgurnus anguillicaudatus*. *Disease Monitoring*, **39**, 78-79.