

Article

Depletion of Albendazole and Its Metabolites and Their Impact on the Gut Microbial Community Following Multiple Oral Dosing in Yellow River Carp (*Cyprinus carpio haematopterus*)

Yue Liu, Yan Dai, Yan-Ni Zhang, Wen-Rui Wang, Yu-Xin Chen, Yang-Guang Jin , Long-Ji Sun, Shi-Hao Li, Fang Yang, Xing-Ping Li  and Fan Yang 

College of Animal Science and Technology, Henan University of Science and Technology, Luoyang 471023, China; lyue0523@126.com (Y.L.); d172580312@126.com (Y.D.); yanni1014@126.com (Y.-N.Z.); wangwenrui1999@163.com (W.-R.W.); z18568335618@126.com (Y.-X.C.); jinyangguang1999@126.com (Y.-G.J.); s17538591074@126.com (L.-J.S.); lishihao0610@126.com (S.-H.L.); yfhaust@126.com (F.Y.)

* Correspondence: lxp@haust.edu.cn (X.-P.L.); fyang@haust.edu.cn or yfscu@126.com (F.Y.)

Abstract

Healthy Yellow River carp (*Cyprinus carpio haematopterus*) reared at a water temperature of 23 ± 0.6 °C were orally administered albendazole (ABZ) at a dose of 12 mg/kg body weight (BW) once daily for seven consecutive days. At predetermined time points after the final administration, five fish were randomly selected for sampling. Plasma, skin-on-muscle, liver, and kidney tissues were collected, and the concentrations of ABZ and its three metabolites—albendazole sulfoxide (ABZSO), albendazole sulfone (ABZSO₂), and albendazole-2-aminosulfone (ABZ-2-NH₂-SO₂)—were determined using high-performance liquid chromatography (HPLC). The results indicated that ABZ and ABZSO were widely distributed across tissues, while ABZSO₂ and ABZ-2-NH₂-SO₂ were only present at trace levels. Pharmacokinetic analysis of ABZ and ABZSO in plasma and tissues was performed using noncompartmental analysis (NCA). ABZ peaked in plasma at 0.73 µg/mL at 24 h after the last administration, with an elimination half-life ($t_{1/2\lambda_Z}$) of 38.56 h. ABZSO reached a peak plasma concentration of 1.54 µg/mL at 24 h, with a $t_{1/2\lambda_Z}$ of 53.73 h. According to China's national standard, where ABZ-2-NH₂-SO₂ is the marker residue with a maximum residue limit (MRL) of 100 µg/kg in fish skin-on muscle, no withdrawal period was necessary. However, based on the European Union standard—which uses the sum of ABZ and its three metabolites as the marker residue and an MRL of 100 µg/kg in ruminants—a withdrawal period of 16 days (or 351 °C-days) was required. Additionally, the study assessed changes in the intestinal microbiota following multiple oral doses of ABZ. The results indicated that ABZ administration significantly altered microbial diversity and composition in a dose- and time-dependent manner. After drug withdrawal, the intestinal microbiota gradually returned to baseline levels, similar to the untreated control group.

Keywords: Yellow River carp; albendazole; metabolites; multiple oral dosing; withdrawal period; gut microbial community

Key Contribution: This study establishes the withdrawal period for albendazole in Yellow River carp after multiple oral dosing, ensuring the safety of consumption by humans. Additionally, the research investigates the effects of albendazole and its metabolites on the intestinal microbiota of the fish, laying the groundwork for future studies on its ecological and health impacts.



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1. Introduction

Fish are a vital source of nutrients and play a key role in global aquaculture production [1]. Their protein composition closely matches human nutritional requirements and is rich in essential amino acids [2,3]. Additionally, fish provide abundant unsaturated fatty acids, vitamins, and minerals [4]. The rising demand for fish has driven aquaculture to become the fastest-growing food-producing sector, contributing approximately 56% of the global food fish supply [5]. According to the Food and Agriculture Organization of the United Nations (FAO), global fisheries and aquaculture production reached a record 223.2 million tons in 2022 [5]. Notably, China remains the only country where aquaculture output exceeds that of capture fisheries, positioning it as a leading producer of aquatic products [5].

The Yellow River carp (*Cyprinus carpio haematopterus*), a prominent aquaculture species in north-central China, is cultivated in the basin of the Yellow River—China's second-longest river, characterized by high sediment loads and rapid water flow [6,7]. As a member of the carp family, the Yellow River carp holds significant nutritional, cultural, and economic importance in China [8]. Its popularity among fish farmers is attributed to its flavorful meat, robust disease resistance, and rapid growth rate [9], making it one of the most widely farmed freshwater species in the region.

Aquaculture is a vital industry in China. However, as production scales expand, parasitic infections have become increasingly common, posing a major challenge to fish farming [10]. To manage these infections, various antiparasitic drugs are routinely applied. Among them, benzimidazole anthelmintics are widely used due to their broad-spectrum antiparasitic efficacy and relatively low toxicity [11]. Albendazole (ABZ), a potent benzimidazole compound, is extensively employed to treat helminth infections in aquaculture [12].

Following administration, ABZ is rapidly metabolized in the liver by microsomal enzymes to its primary active metabolite, albendazole sulfoxide (ABZ-SO), which is responsible for its anthelmintic activity but is also associated with certain toxicological effects [13]. ABZ-SO is further oxidized to an inactive metabolite, albendazole sulfone (ABZ-SO₂), which lacks anthelmintic properties [13]. Subsequently, ABZ-SO₂ may undergo deacetylation of the carbamate group, forming another inactive and highly polar metabolite, albendazole-2-aminosulfone (ABZ-2-NH₂-SO₂) [14].

Toxicological studies have demonstrated that both ABZ and its active metabolite ABZ-SO can be teratogenic in farm animals and laboratory models [15]. Consumption of animal-derived foods containing ABZ and its metabolite residues exceeding the established maximum residue limit (MRL) may pose health risks to consumers [16]. For instance, excessive ABZ-SO residues have been linked to increased oxidative stress and hepatocellular damage, potentially resulting in liver dysfunction [17]. In pregnant women, exposure to ABZ residues may allow the drug to cross the placental barrier, increasing the risk of birth defects or miscarriage [18].

China is one of the few countries that have approved the use of ABZ in aquaculture and has established corresponding MRL values. Specifically, China has set an MRL of 100 µg/kg for ABZ in fish muscle with skin, using ABZ-2-NH₂-SO₂ as the marker residue [19]. In contrast, while both the United States and the European Union (EU) have established MRLs for ABZ, these limits are primarily based on data from terrestrial animals, particularly ruminants, with limited application to aquatic species. For example, EU Regulation No. 37/2010 specifies an MRL of 100 µg/kg for ABZ residues in ruminant muscle, expressed as the sum of ABZ and its three major metabolites (ABZ-SO, ABZ-SO₂, and ABZ-2-NH₂-SO₂) [20].

The intestine plays a vital role in animal growth and development, functioning not only as a site for digestion and nutrient absorption but also as a protective barrier against harmful substances [21]. Intestinal microorganisms are deeply involved in host metabolic

processes and are essential for maintaining gut homeostasis [22]. The gut microbiota has been identified as a key environmental factor influencing digestion, nutrient uptake, and overall physiological metabolism in animals [23]. In fish, the establishment of a healthy and balanced gut microbiota is crucial for maintaining health, primarily through mechanisms such as competitive exclusion. Moreover, the gut microbiota contributes significantly to the development and maturation of the immune system, particularly the innate immune response, which is essential for disease resistance [24].

Previous studies have demonstrated that ABZ is effective against a variety of endoparasites in aquaculture, such as monogeneans [25–27], *Loma salmonae* [28], and *Neoechinorhynchus buttnerae* [29], and it is widely used due to its broad-spectrum antiparasitic activity [12]. Pharmacokinetic and residue studies in fish species such as tambaqui [30], rainbow trout [15], tilapia [15], Atlantic salmon [15], and Yellow River carp [31] have shown that ABZ and its primary metabolite albendazole sulfoxide (ABZSO) are rapidly absorbed and eliminated, although tissue distribution patterns and elimination rates may vary depending on the species, temperature, and dosage regimen. Regarding toxicity, existing studies have reported that although ABZ has a wide safety margin in fish, high or prolonged exposure may affect liver function and induce oxidative stress [32]. However, research on the gut microbiota response to ABZ exposure in fish is still extremely limited. Despite the established pharmacological profile of ABZ in several fish species, there is a notable gap in understanding how ABZ administration affects gut microbial composition, which plays a crucial role in fish health, nutrient absorption, and immune responses. Moreover, limited data are available regarding ABZ residue depletion and withdrawal period in Yellow River carp (*Cyprinus carpio haematopterus*), a widely farmed native Chinese species.

Therefore, the objectives of this study were twofold: (1) to establish the withdrawal period of ABZ after multiple oral doses in Yellow River carp, and (2) to evaluate the potential impact of repeated ABZ administration on the composition of the gut microbial community.

2. Materials and Methods

2.1. Drugs and Chemical Reagents

The reference standard for ABZ (Lot No. H0901807; 100% purity) was obtained from the China Institute of Veterinary Drug Control (Beijing, China). The standard for ABZ-SO (Lot No. Y06D6C6698; 98% purity) was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Standards for ABZ-SO₂ (Lot No. LA50 × 123; 98% purity) and ABZ-2-NH₂-SO₂ (Lot No. L280V99; 98% purity) were obtained from Bailiwick Technology Co., Ltd. (Shanghai, China). The internal standard, oxibendazole (OBZ; Lot No. L365794; 99% purity), was provided by Shanghai Haohong Biopharmaceutical Technology Co., Ltd. (Shanghai, China). Albendazole powder (6 g/100 g; Lot No. 120369001) was purchased from Anhui Hengyuan Pharmaceutical Co., Ltd. (Bengbu, Anhui, China).

Ethyl acetate was purchased from Shanghai Eon Chemical Technology Co., Ltd. (Shanghai, China). Chromatography-grade methanol and acetonitrile were supplied by Shanghai Maclean Biochemical Technology Co., Ltd. (Shanghai, China). Dimethyl sulfoxide, sodium metabisulfite, glacial acetic acid, sodium hydroxide, hexane, and potassium carbonate were obtained from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China).

The PCR product purification kit (Universal DNA Purification and Recovery Kit) was obtained from Tiangen Biotech Co., Ltd. (Beijing, China). Phusion® High-Fidelity PCR Master Mix with GC Buffer was purchased from New England Biolabs (Ipswich, MA, USA).

2.2. Animals

Forty healthy Yellow River carp (*Cyprinus carpio haematopterus*) with an average BW of 0.73 ± 0.085 kg (range: 0.53–1.02 kg) were used for the residue depletion study. All fish were

obtained from Xingda Aquaculture Co., Ltd. (Zhengzhou, Henan, China). Five fish served as the control group, providing blank samples for further quantification, and the other fish were randomly and equally divided into seven groups and placed in numbered net cages (0.5 m × 0.5 m × 0.65 m). These cages were distributed in fiberglass tanks (approximately 650 L; 1.3 m × 0.8 m × 0.65 m), with one cage per tank. The water temperature was maintained at 23 ± 0.6 °C using a thermostatic heating rod. The water quality parameters remained stable, and the fish were fed drug-free commercial feed daily.

An additional 30 healthy Yellow River carp with an average BW of 54.2 g (range: 41.7–63.2 g) were used for gut microbiota analysis. These fish were also obtained from Xingda Aquaculture Co., Ltd. (Zhengzhou, Henan, China) and were randomly and equally divided into six groups. Each group was reared in a separate fiberglass tank of the same dimensions and volume as described above, with water temperature consistently maintained at 23 ± 0.6 °C.

All experimental procedures were conducted in accordance with the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Henan University of Science and Technology (Approval No. 20240302).

2.3. Residue Depletion of Albendazole and Its Metabolites

2.3.1. Administration and Sampling

The 40 healthy carp were randomly and equally divided into 8 groups. One group served as the untreated control and was used to provide blank plasma and tissue samples for subsequent quantification. The remaining seven groups received oral administration of ABZ at a dose of 12 mg/kg body weight (BW) once daily for seven consecutive days. This dosage was selected based on the recommended therapeutic range stated in the product instructions for the commercial albendazole powder formulation approved for use in aquaculture. The administered 7-day regimen corresponds to the recommended therapeutic practice using 6% albendazole powder for freshwater fish in China, targeting monogenean parasites such as *Dactylogyrus* and *Trichodina* spp., which commonly infect carp species.

Albendazole powder was accurately weighed and thoroughly mixed with an appropriate volume of 1% sodium carboxymethyl cellulose (CMC-Na) to prepare a uniform suspension containing 12 mg/mL of ABZ, which was then administered via gavage. This suspension was freshly prepared each day prior to administration. Healthy Yellow River carp (*Cyprinus carpio haematopterus*) were used in this study, which maintained normal vitality throughout the 7-day consecutive oral gavage administration period. Moreover, the carp remained highly active until the sampling time point.

At 0.5, 1, 2, 3, 5, 10, and 15 days after the final dose, one group of fish was randomly selected at each time point for sampling. The selection of these sampling times and the number of fish per group were based on pharmacokinetic data obtained from our previous laboratory study [31]. Approximately 1 mL of blood was collected from the caudal vein of each fish into tubes containing heparin sodium as an anticoagulant. No anesthetic was used during blood sampling in order to avoid potential interference with the pharmacokinetics and disposition of albendazole and its metabolites. Following blood collection, the fish were euthanized by a blow to the head, and samples of skin-on muscle, liver, and kidney were collected. Blood samples were centrifuged at $4000 \times g$ for 10 min to obtain plasma. All collected samples were stored at -20 °C until analysis.

2.3.2. Analytical Methods

A previously validated chromatographic method was employed to quantify the concentrations of ABZ and its three metabolites in plasma and tissue samples [23]. Detailed

procedures for sample pretreatment can be found in a previously published study from our laboratory [31].

Chromatographic separation was performed using a Hypersil BDS C18 column (5 μm ; 250 $\times$ 4.6 mm i.d.; Dalian Elite Analytical Instrument Co., Ltd., Dalian, Liaoning, China), with the column temperature maintained at 30 $^{\circ}\text{C}$. The external standard method was used to quantify ABZ and its metabolites in plasma, as well as metabolites in tissue samples. In contrast, ABZ in tissue samples was quantified using the internal standard method, with OBZ serving as the internal standard.

The mobile phase consisted of acetonitrile, methanol, and 0.05 mol/L ammonium acetate (pH adjusted to 5.0 with glacial acetic acid). For plasma analysis, the mobile phase ratio was 30:15:55 for ABZ and 18:7:75 for its metabolites. For tissue samples, the ratios were 40:10:50 for ABZ and 23:10:67 for its metabolites. The flow rate was set at 1.0 mL/min for all analyses, and all injection volumes were 20 μL .

2.3.3. Data Analysis

Concentrations of ABZ and its metabolites in all samples at each time point were expressed as mean $\pm$ standard deviation (SD). Tissue distribution analysis was performed using non-compartmental analysis (NCA) with Phoenix software (version 8.1; Pharsight, Northampton, MA, USA) to calculate the pharmacokinetic parameters following multiple oral doses of ABZ. Elimination curves were generated using Origin software (version 9.0; OriginLab, Northampton, MA, USA).

According to Chinese regulations, the MRL for ABZ in fish skin-on muscle is set at 100 $\mu\text{g}/\text{kg}$, with ABZ-2-NH₂-SO₂ designated as the marker residue. In the present study, following oral administration of ABZ at 12 mg/kg BW per day for seven consecutive days, the concentrations of ABZ-2-NH₂-SO₂ in all skin-on muscle samples remained consistently below the established MRL. Therefore, based on the current Chinese MRL standard and the observed residue levels, a withdrawal period for ABZ under this dosing regimen is not required.

However, to further evaluate residue depletion from a regulatory perspective, we also applied the marker residue definition adopted by the EU, which is the sum of ABZ and its three metabolites (ABZ-SO, ABZ-SO₂, and ABZ-2-NH₂-SO₂), with an MRL of 100 $\mu\text{g}/\text{kg}$ in skin-on muscle. It should be noted that this marker residue and corresponding MRL were originally established for ruminants, not aquatic species. To calculate the withdrawal period, an elimination curve was constructed based on the total concentrations of ABZ and its three metabolites in skin-on muscle. The analysis was performed using WT 1.4 software, with a 95% confidence level, ensuring the 99th percentile of the tolerance limit (MRL). Prior to withdrawal time calculation, the dataset was evaluated using WT 1.4 to verify compliance with the four underlying assumptions required for linear regression: (1) linearity of loge (concentration) versus time, (2) independence of observations, (3) normality of residuals on a log scale, and (4) homoscedasticity [33].

2.4. Effects on the Gut Microbial Community

2.4.1. Administration and Sampling

A total of 30 healthy Yellow River carp were randomly and equally assigned to five groups: one control group (KB) and four treatment groups (A1, A2, B1, and B2). The control group received no treatment, while the four treatment groups were administered ABZ orally at a dose of 12 mg/kg BW once daily. All ABZ administrations were conducted between 8:00 and 9:00 a.m. daily. For microbiota analysis, intestinal content samples were collected from groups A1 and A2 at approximately 5:00 p.m. on days 4 and 7 of ABZ administration, respectively. Samples from groups B1 and B2 were collected at approximately 5:00 p.m. on

days 1 and 5 following the completion of seven consecutive days of ABZ administration. The KB was sampled concurrently with group B2. Intestinal contents were collected using sterile instruments and immediately stored at -80°C until further analysis.

2.4.2. DNA Extraction and PCR Amplification of Intestinal Samples

Genomic DNA was extracted from intestinal content samples using the cetyltrimethylammonium bromide (CTAB) method. DNA purity and concentration were assessed by 1% agarose gel electrophoresis. An appropriate amount of DNA was transferred to a centrifuge tube and diluted to a final concentration of $1\text{ ng}/\mu\text{L}$ using sterile water.

Polymerase chain reaction (PCR) amplification was performed targeting the V3–V4 hypervariable region of the bacterial 16S rRNA gene using the primer pair 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGGTATCTAAT-3').

2.4.3. Purification and Mixing of PCR Products

PCR products from each sample were quantified and then mixed in equal amounts based on their concentrations. The mixed PCR products were purified using 2% agarose gel electrophoresis in $1\times$ TAE buffer. Target DNA bands were excised from the gel and recovered using the Universal DNA Purification and Recovery Kit (Tiangen Biotech Co., Ltd., Beijing, China), following the manufacturer's instructions.

2.4.4. Library Construction, On-Line Sequencing and Information Analysis

Library construction was carried out using the NEBNext[®] Ultra DNA Library Prep Kit (New England Biolabs, Ipswich, MA, USA), following the manufacturer's protocol. The quality and concentration of the constructed libraries were assessed using quantitative PCR (qPCR) on an Agilent 5400 system. Libraries that passed quality control were subjected to high-throughput sequencing on the Illumina platform.

Raw sequencing data (FASTQ format) were imported into QIIME2 (version 2024.2) using the qiime tools import plugin. Subsequent bioinformatic analyses, including assessments of alpha and beta diversity, microbial community composition, and relative abundance, were performed using QIIME2 software.

3. Results

3.1. Residue Depletion of Albendazole and Its Metabolites

3.1.1. Residue Concentrations of ABZ and Its Three Metabolites

Throughout the study, all fish remained in good health, and no adverse effects were observed following ABZ administration. Post-mortem examination revealed no visible abnormalities in any tissues or organs. Furthermore, ABZ and its three metabolites were not detected in any samples collected from untreated (control) Yellow River carp, confirming the absence of background contamination.

The mean concentrations of ABZ and its three metabolites in plasma, skin-on muscle, liver, and kidney tissues after multiple oral doses are presented in Tables 1–4, and Figures 1–3 show the concentration–time profiles of ABZ, ABZSO, and ABZSO₂ in the collected tissues. In contrast, ABZ-2-NH₂–SO₂ was detected only sporadically in skin-on muscle and kidney samples, with considerable variability in its concentration–time data. Due to this irregularity, a concentration–time curve for ABZ-2-NH₂–SO₂ was not generated.

Overall, ABZ and its metabolites were distributed in most collected tissues, demonstrating extensive systemic distribution in Yellow River carp following repeated oral administration.

Table 1. Mean $\pm$ SD concentrations ($\mu\text{g/g}$ or $\mu\text{g/mL}$) of ABZ in plasma, skin-on muscle, liver, and kidney samples collected from Yellow River carp ($n = 5$ fish per time point) following multiple oral doses of ABZ at a dose of 12 mg/kg BW per day for seven consecutive days.

Time (h)	Plasma	Skin-Muscle	Liver	Kidney
12	0.298 ± 0.135	0.442 ± 0.231	1.094 ± 0.336	2.164 ± 0.892
24	0.733 ± 0.250	0.624 ± 0.344	0.656 ± 0.420	1.287 ± 0.315
48	0.444 ± 0.062	0.282 ± 0.153	0.335 ± 0.080	0.880 ± 0.188
72	0.236 ± 0.154	0.172 ± 0.052	0.128 ± 0.086	0.245 ± 0.178
120	0.048 ± 0.013	0.061 ± 0.057	1.094 ± 0.336	0.035 ± 0.023
240	ND	0.012 ± 0.006	ND	ND
360	ND	ND	ND	ND

ND = Not detectable.

Table 2. Mean $\pm$ SD concentrations ($\mu\text{g/g}$ or $\mu\text{g/mL}$) of ABZSO in plasma, skin-on muscle, liver, and kidney samples collected from Yellow River carp ($n = 5$ fish per time point) following multiple oral doses of ABZ at a dose of 12 mg/kg BW per day for seven consecutive days.

Time (h)	Plasma	Skin-Muscle	Liver	Kidney
12	1.203 ± 0.103	1.479 ± 0.115	ND	ND
24	1.559 ± 0.327	2.198 ± 0.277	4.145 ± 1.759	2.455 ± 0.512
48	1.397 ± 0.187	1.334 ± 0.160	3.296 ± 0.574	3.347 ± 0.704
72	0.691 ± 0.245	0.702 ± 0.128	2.023 ± 0.366	1.867 ± 0.236
120	0.545 ± 0.174	0.466 ± 0.237	1.221 ± 0.535	1.039 ± 0.333
240	0.078 ± 0.022	0.076 ± 0.028	0.304 ± 0.086	0.394 ± 0.085
360	ND	ND	ND	ND

ND = Not detectable.

Table 3. Mean $\pm$ SD concentrations ($\mu\text{g/g}$ or $\mu\text{g/mL}$) of ABZSO₂ in plasma, skin-on muscle, liver, and kidney samples collected from Yellow River carp ($n = 5$ fish per time point) following multiple oral doses of ABZ at a dose of 12 mg/kg BW per day for seven consecutive days.

Time (h)	Plasma	Skin-Muscle	Liver	Kidney
12	0.038 ± 0.006	0.069 ± 0.011	ND	ND
24	0.022 ± 0.007	0.042 ± 0.008	0.270 ± 0.056	0.103 ± 0.020
48	0.037 ± 0.012	0.052 ± 0.012	0.194 ± 0.057	0.127 ± 0.070
72	0.018 ± 0.003	0.031 ± 0.005	0.304 ± 0.131	0.106 ± 0.043
120	0.015 ± 0.002	0.030 ± 0.004	0.149 ± 0.018	0.106 ± 0.033
240	0.011 ± 0.002	0.020 ± 0.003	0.066 ± 0.028	0.057 ± 0.014
360	ND	ND	ND	ND

ND = Not detectable.

Table 4. Mean $\pm$ SD concentrations ($\mu\text{g/g}$ or $\mu\text{g/mL}$) of ABZ-2-NH₂-SO₂ in plasma, skin-on muscle, liver, and kidney samples collected from Yellow River carp ($n = 5$ fish per time point) following multiple oral doses of ABZ at a dose of 12 mg/kg BW per day for seven consecutive days.

Time (h)	Plasma	Skin-Muscle	Liver	Kidney
12	ND	0.0748 ± 0.0078	ND	ND
24	ND	0.0402 ± 0.0114	ND	0.0655 ± 0.0351
48	ND	0.0757 ± 0.0257	ND	0.0350 ± 0.0104
72	ND	0.0655 ± 0.0137	ND	0.0418 ± 0.0201
120	ND	0.0542 ± 0.0191	ND	0.0669 ± 0.0515
240	ND	0.0234 ± 0.0024	ND	ND
360	ND	ND	ND	ND

ND = Not detectable.

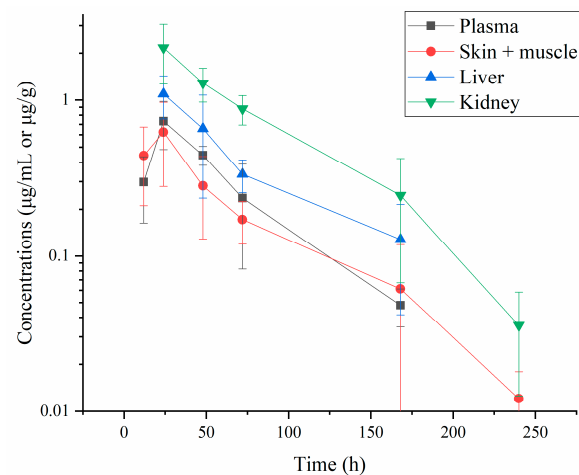


Figure 1. Mean $\pm$ SD concentrations ($\mu\text{g/g}$ or $\mu\text{g/mL}$) of ABZ in plasma, skin-on muscle, liver, and kidney samples collected from Yellow River carp ($n = 5$ fish per time point) following multiple oral doses of ABZ at a dose of 12 mg/kg BW per day for seven consecutive days.

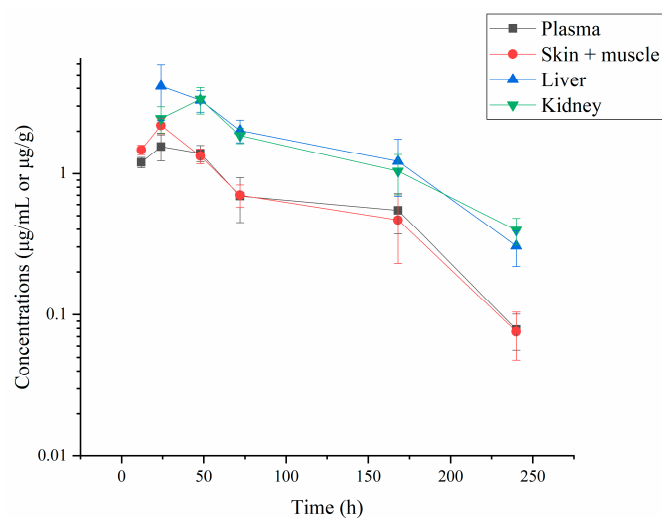


Figure 2. Mean $\pm$ SD concentrations ($\mu\text{g/g}$ or $\mu\text{g/mL}$) of ABZSO in plasma, skin-on muscle, liver, and kidney samples collected from Yellow River carp ($n = 5$ fish per time point) following multiple oral doses of ABZ at a dose of 12 mg/kg BW per day for seven consecutive days.

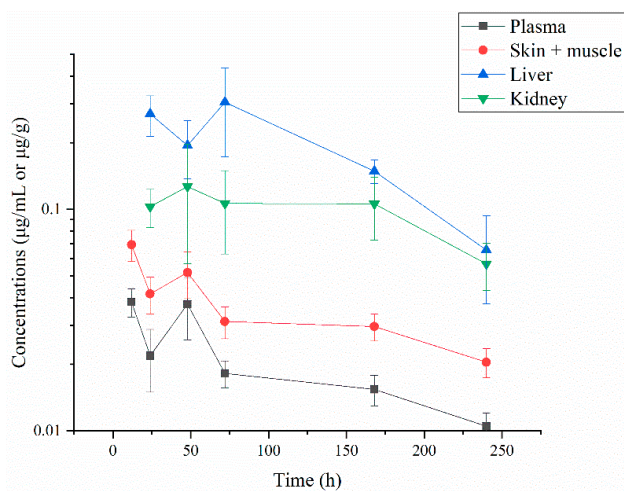


Figure 3. Mean $\pm$ SD concentrations ($\mu\text{g/g}$ or $\mu\text{g/mL}$) of ABZSO₂ in plasma, skin-on muscle, liver, and kidney samples collected from Yellow River carp ($n = 5$ fish per time point) following multiple oral doses of ABZ at a dose of 12 mg/kg BW per day for seven consecutive days.

3.1.2. Residue Depletion of ABZ and Its Three Metabolites

Due to the low and irregular concentrations of ABZSO₂ and ABZ-2-NH₂-SO₂ in most tissues, only ABZ and ABZSO were included in the pharmacokinetic analysis. The primary pharmacokinetic parameters derived using NCA are summarized in Tables 5 and 6. Following the final oral dosing, the plasma concentration of ABZ reached a peak ($C_{\max}$) of 0.73 µg/mL at 24 h. Similarly, ABZSO reached a maximum plasma concentration of 1.54 µg/mL at 24 h. The elimination half-life ($t_{1/2\lambda_z}$) of ABZ across different tissues was as follows: liver (55.04 h) > skin-on muscle (44.77 h) > plasma (38.56 h) > kidney (38.53 h). For ABZSO, the order of $t_{1/2\lambda_z}$ was kidney (77.46 h) > liver (61.74 h) > skin-on muscle (54.19 h) > plasma (53.73 h). The area under the concentration–time curve (AUC) values for ABZ were 40.12, 46.49, 77.96, and 160.03 h·µg/g (or h·µg/mL) in skin-on muscle, plasma, liver, and kidney, respectively. Corresponding AUC values for ABZSO were 185.60, 175.94, 458.89, and 403.47 h·µg/g (or h·µg/mL), respectively.

Table 5. Pharmacokinetic parameters of ABZ in plasma, skin-on muscle, liver, and kidney samples collected from Yellow River carp ($n = 5$ fish per time point) following multiple oral doses of ABZ at a dose of 12 mg/kg BW per day for seven consecutive days.

Parameters	Unit	Plasma	Skin-on Muscle	Liver	Kidney
λ_z	1/h	0.018	0.015	0.013	0.018
$t_{1/2\lambda_z}$	h	38.56	44.77	55.04	38.53
$T_{\max}$	h	24.00	24.00	24.00	24.00
$C_{\max}$	µg/mL or µg/g	0.73	0.62	1.09	2.16
$AUC_{0-\infty}$	h·µg/mL or h·µg/g	46.49	40.12	77.96	160.03
$AUMC_{0-\infty}$	h ² ·µg/mL or h ² ·µg/g	2861.11	2654.70	6274.72	11,038.61
MRT	h	61.54	66.16	80.48	68.98
AUC%	%	5.62	2.34	12.50	1.57

λ_z , first-order rate constant associated with the terminal elimination phase; $t_{1/2\lambda_z}$, terminal half-life; $T_{\max}$, time to reach peak concentration; $C_{\max}$, peak concentration; $AUC_{0-\infty}$, area under the drug concentration–time curve from the time of administration to infinity; $AUMC_{0-\infty}$, area under first-order moment curve from administration to infinity; MRT, mean residence time; AUC%, the percentage of the area under the curve extrapolated from the last quantifiable concentration to infinity.

Table 6. Pharmacokinetic parameters of ABZSO in plasma, skin-on muscle, liver, and kidney samples collected from Yellow River carp ($n = 5$ fish per time point) following multiple oral doses of ABZ at a dose of 12 mg/kg BW per day for seven consecutive days.

Parameters	Unit	Plasma	Skin-on Muscle	Liver	Kidney
λ_z	1/h	0.013	0.013	0.011	0.0090
$t_{1/2\lambda_z}$	h	53.73	54.19	61.74	77.46
$T_{\max}$	h	24.00	24.00	24.00	48.00
$C_{\max}$	µg/mL or µg/g	1.54	2.29	4.51	3.35
$AUC_{0-\infty}$	h·µg/mL or h·µg/g	175.94	185.60	458.89	403.47
$AUMC_{0-\infty}$	h ² ·µg/mL or h ² ·µg/g	16,687.22	15,934.63	46,240.91	49,205.87
MRT	h	94.85	85.85	100.77	121.96
AUC%	%	4.97	4.68	7.31	12.48

λ_z , first-order rate constant associated with the terminal elimination phase; $t_{1/2\lambda_z}$, terminal half-life; $T_{\max}$, time to reach peak concentration; $C_{\max}$, peak concentration; $AUC_{0-\infty}$, area under the drug concentration–time curve from the time of administration to infinity; $AUMC_{0-\infty}$, area under first-order moment curve from administration to infinity; MRT, mean residence time; AUC%, the percentage of the area under the curve extrapolated from the last quantifiable concentration to infinity.

3.1.3. Withdrawal Period of ABZ After Multiple Oral Dosing

Based on the marker residue ABZ-2-NH₂-SO₂ and its MRL of 100 µg/kg established by Chinese regulatory authorities, no withdrawal period is required for ABZ in Yellow River carp after oral administration of 12 mg/kg BW per day for seven consecutive days,

as all detected concentrations of ABZ-2-NH₂-SO₂ in skin-on muscle were below the MRL (Table 4).

However, when applying the EU standard, which defines the marker residue as the sum of ABZ and its three metabolites (ABZ-SO, ABZ-SO₂, and ABZ-2-NH₂-SO₂) with an MRL of 100 µg/kg for ruminant muscle, the withdrawal time was calculated using WT 1.4 software. The estimated withdrawal period was 15.23 days (Figure 4), which was rounded up to 16 days in accordance with European Medicines Agency (EMA) guidelines. When expressed as degree-days (°C-days), the withdrawal period corresponded to 351 °C-days (15.23 days × 23 °C).

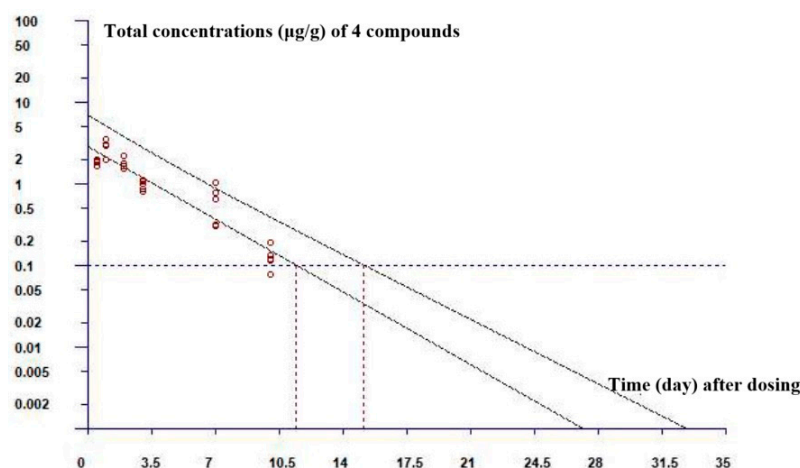


Figure 4. Semi-logarithmic elimination curves of ABZ and its three metabolites in skin-on muscle of Yellow River carp reared at 23 ± 0.6 °C following multiple oral administrations of ABZ at 12 mg/kg BW per day for seven consecutive days. The solid lines represent the depletion regression line and the upper 95% confidence limit. Dots indicate the measured concentrations at each time point. The horizontal dashed line denotes the EU MRL of 100 µg/kg for the marker residue, defined as the sum of ABZ, ABZ-SO, ABZ-SO₂, and ABZ-2-NH₂-SO₂.

3.2. Gut Microbial Community Test

3.2.1. Effects of ABZ Oral Administration on the Gut Microbial Community of Yellow River Carp

As illustrated in Figure 5, multiple oral dosing of ABZ significantly altered the composition of the dominant intestinal microbiota in Yellow River carp at both the phylum and genus levels. At the phylum level (Figure 5a), the gut microbiota of all groups—KB (control), A1, A2, B1, and B2—was composed mainly of *Pseudomonadota*, *Bacillota*, *Fusobacteriota*, *Bacteroidota*, *Actinomycetota*, and several unclassified bacterial taxa. The relative abundances of *Pseudomonadota* were 55.73%, 35.67%, 33.52%, 27.98%, and 43.91%, respectively, in the KB, A1, A2, B1, and B2 groups. Corresponding values for *Bacillota* were 27.94%, 31.46%, 33.57%, 40.92%, and 29.89%; for *Fusobacteriota*: 2.35%, 11.24%, 13.51%, 10.76%, and 9.16%; for *Bacteroidota*: 4.26%, 11.62%, 10.36%, 6.84%, and 5.81%; and for *Actinomycetota*: 6.73%, 7.21%, 5.29%, 8.40%, and 6.55%, respectively.

These results indicate that the relative abundance of *Pseudomonadota* decreased with continued ABZ administration and gradually recovered after drug withdrawal. In contrast, the abundance of *Bacillota* increased with higher ABZ exposure and remained elevated even after treatment cessation. *Fusobacteriota*, *Bacteroidota*, and *Actinomycetota* also showed dose-dependent increases in relative abundance, which declined following withdrawal.

At the genus level (Figure 5b), the relative abundance of *g_Peptostreptococcaceae_Incertae_Sedis* in the KB, A1, A2, B1, and B2 groups was 1.03%, 12.21%, 4.61%, 13.12%, and 10.39%, respectively. The corresponding values for *Aeromonas* were 0.28%, 2.88%, 3.29%, 1.23%, and 4.19%. These findings suggest that the relative abundance of both *g_Peptostreptococcaceae_Incertae_Sedis*

and *Aeromonas* increased following ABZ administration and did not return to baseline levels even after drug withdrawal. The relative abundance of *Bacillus* spp. in KB, A1, A2, B1, and B2 groups was 2.79%, 3.06%, 4.57%, 6.69%, and 4.83%, respectively. This trend shows an increase in *Bacillus* spp. with escalating ABZ exposure, followed by a gradual decline post-treatment.

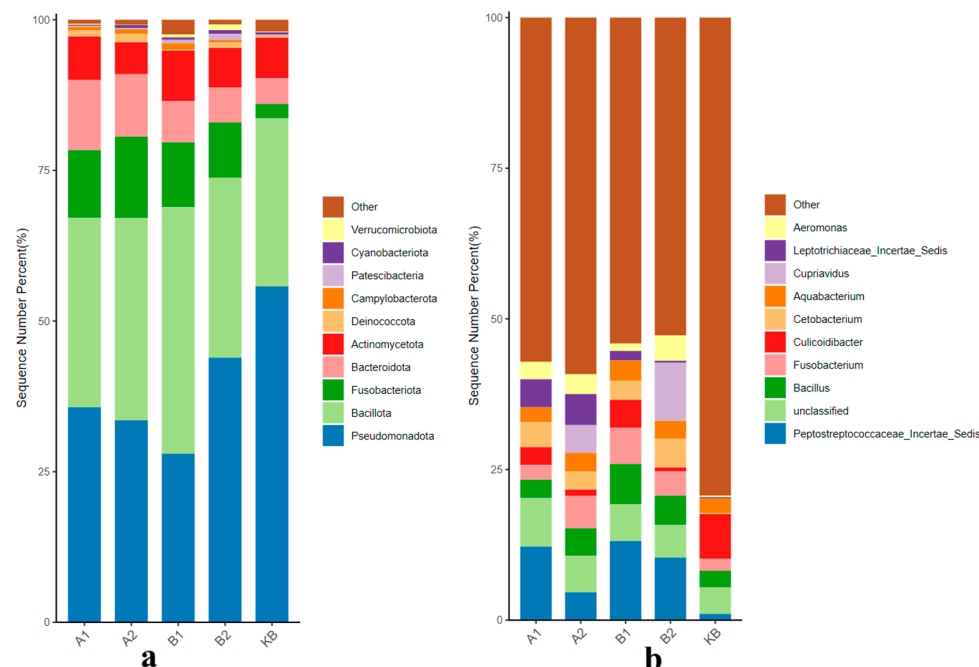


Figure 5. Composition of intestinal microbiota at the phylum and genus levels in Yellow River carp (*Cyprinus carpio haematopterus*) at different time points following ABZ administration. (a) Phylum lever; (b) genus lever.

The KB received no ABZ treatment and served as the control. Groups A1 and A2 were orally administered ABZ at a dose of 12 mg/kg BW per day for 4 and 7 consecutive days, respectively, and sampled on the day of administration. Groups B1 and B2 were sampled on Days 1 and 5 after completing 7 consecutive days of ABZ administration.

3.2.2. Differential Analysis of ABZ Oral Administration on Intestinal Bacterial Marker Species in Yellow River Carp

As shown in Figure 6, prior to ABZ administration, *Alphaproteobacteria* (within the phylum *Ascomycota*), *Caulobacteraceae* (within the order *Stalkyobacteria*), and *Brevundimonas* exhibited LDA scores greater than 3 in the control group (KB), indicating that these taxa contributed significantly to the differences in bacterial community structure among the groups. Following 4 and 7 days of ABZ administration (groups A1 and A2), *Bacteroidia* (phylum *Bacteroidota*) displayed an LDA score greater than 4, suggesting a dominant role in shaping group-level microbial differences during treatment. After 1 day of drug withdrawal (group B1), *Paracoccus* species emerged as key contributors to microbial structural variation. By Day 5 post-treatment (group B2), *Verrucomicrobiota* and *Luteolibacter* species showed LDA scores greater than 4, indicating their significant influence on the gut microbiota at this recovery stage.

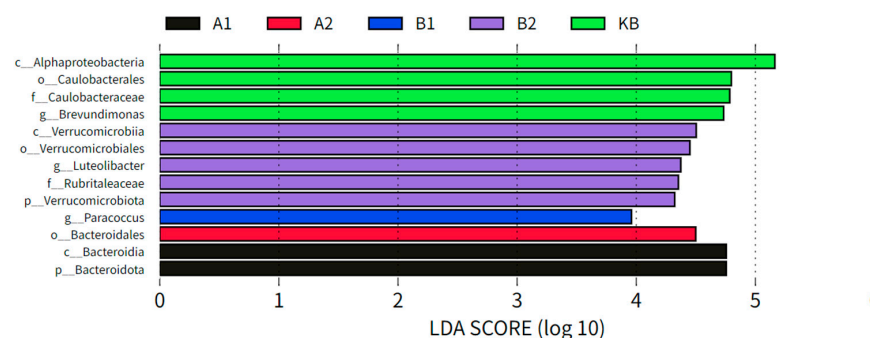


Figure 6. LefSe analysis of the differential intestinal microbiota in Yellow River carp (*Cyprinus carpio haematopterus*) following ABZ administration. Taxa with linear discriminant analysis (LDA) scores >3.0 are shown, indicating significantly enriched microbial clades contributing to differences among treatment groups at various time points before, during, and after ABZ exposure.

3.2.3. Effects of ABZ Oral Administration on the Number and Diversity of Intestinal Microbial OTUs in Yellow River Carp

After processing and analyzing the raw sequencing data, the number of operational taxonomic units (OTUs) identified in the intestinal samples of Yellow River carp is presented in Figure 7. As shown in Figure 7a, a total of 911 OTUs were observed in the KB (untreated control). In contrast, 804 and 659 OTUs were detected in groups A1 and A2 after 4 and 7 days of continuous ABZ administration, respectively, indicating a notable decrease in OTU richness with prolonged drug exposure. Figure 7b shows that 499 and 591 OTUs were detected in groups B1 and B2 at 1 and 5 days after drug withdrawal, respectively. Although the OTU numbers increased slightly following cessation of treatment, they remained substantially lower than the control group value of 911 OTUs, suggesting a slow and incomplete recovery of microbial diversity.

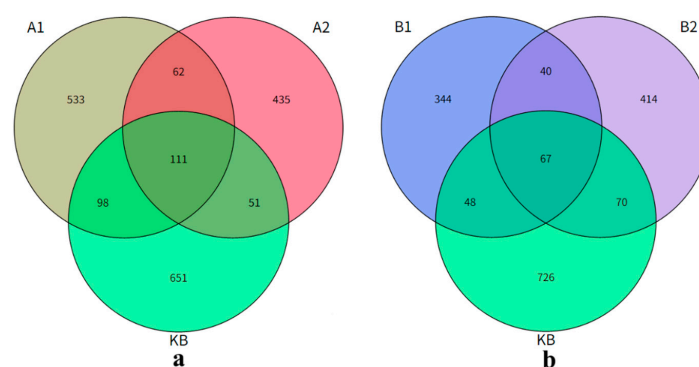


Figure 7. Number of operational taxonomic units (OTUs) in intestinal samples of Yellow River carp (*Cyprinus carpio haematopterus*) at different time points before, during, and after ABZ administration. (a) OTU counts in control (KB) and treated groups (A1 and A2) after 4 and 7 days of ABZ exposure. (b) OTU counts in withdrawal groups (B1 and B2) at 1 and 5 days after cessation of ABZ treatment.

Principal coordinate analysis (PCoA) of the intestinal microbial communities in Yellow River carp is presented in Figure 8. As shown in Figure 8a, groups A1 and A2, which received ABZ treatment, were distinctly separated from the control KB along the primary coordinate (Axis.1), indicating that ABZ administration significantly altered the gut microbiota composition. However, the spatial separation between A1 and A2 was minimal, suggesting no substantial difference in microbial communities between the two time points during treatment. Figure 8b illustrates a gradual shift in the microbial composition of groups B1 and B2 toward the KB following cessation of ABZ administration, indicating a partial recovery of the intestinal microbiota over time.

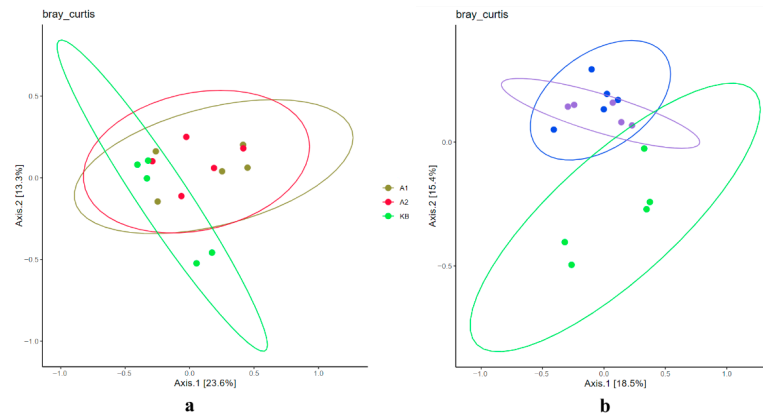


Figure 8. Principal coordinate analysis (PCoA) of the intestinal microbiota in Yellow River carp (*Cyprinus carpio haematopterus*). (a) Microbial community shifts in response to ABZ administration. Groups A1 and A2, sampled on Days 4 and 7 of ABZ treatment, respectively, show clear separation from the untreated control group (KB) along the primary coordinate axis, indicating ABZ-induced alterations in gut microbiota. (b) Microbial community structure in post-treatment groups B1 and B2 (sampled at Days 1 and 5 after ABZ withdrawal, respectively) shows a gradual return toward the control group, suggesting partial recovery of the gut microbial composition.

The effects of ABZ on the intestinal microbial diversity of Yellow River carp were evaluated using the Chao1, Faith's phylogenetic diversity, and Observed species indices, as presented in Figure 9. The results revealed that all three diversity indices—Chao1 (Figure 9a), Faith's index (Figure 9b), and Observed species (Figure 9c)—significantly decreased with the duration of ABZ administration, indicating a loss of microbial richness and phylogenetic diversity. Following cessation of ABZ treatment, a partial rebound in all indices was observed, although they remained lower than those of the untreated control group (KB). These findings suggest that ABZ exposure markedly disrupts the gut microbial diversity of Yellow River carp (*Cyprinus carpio haematopterus*), with only limited recovery observed after drug withdrawal.

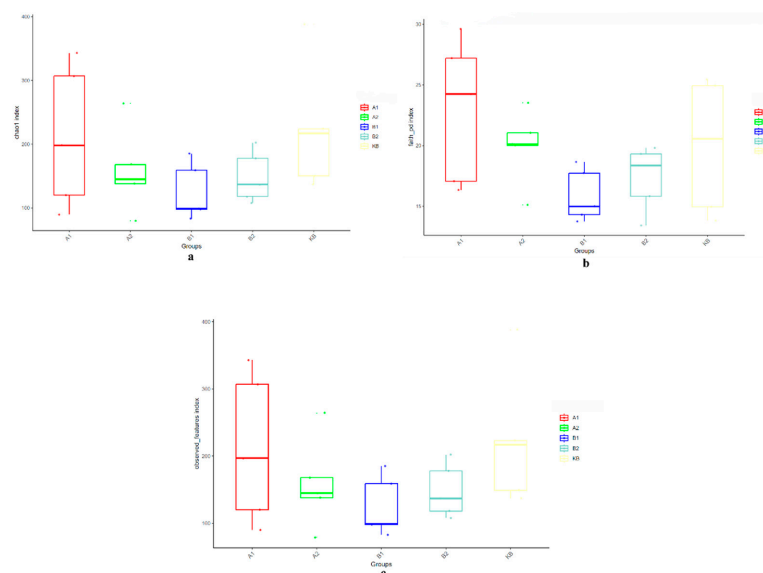


Figure 9. Effects of ABZ administration on gut microbial diversity in Yellow River carp (*Cyprinus carpio haematopterus*), as evaluated by Chao1, Faith's phylogenetic diversity, and Observed species indices. (a) Chao1 index, (b) Faith's phylogenetic diversity index, and (c) Observed species index. All three indices decreased following ABZ administration (groups A1 and A2) and partially recovered after cessation of treatment (groups B1 and B2), compared to the untreated control group (KB), indicating that ABZ administration reduced intestinal microbial diversity in a time-dependent manner.

4. Discussion

In this study, after repeated oral administration of ABZ at 12 mg/kg body weight (BW) for seven consecutive days in Yellow River carp, neither ABZ nor its metabolite ABZ-SO exhibited a complex multi-peak phenomenon in plasma concentration–time profiles. This contrasts markedly with the profiles observed following a single dose [31]. The absence of multiple peaks in the present study may be attributed to the sampling schedule. The relatively sparse sampling points and extended intervals may have limited the resolution of the pharmacokinetic curves. A denser sampling strategy, especially during the early post-administration period, would likely provide a more accurate characterization of drug concentration dynamics following repeated dosing.

Following multiple oral administrations, the $C_{\max}$ of ABZ-SO in skin-on muscle reached 2.29 $\mu\text{g/g}$ at 24 h, with a $t_{1/2\lambda_z}$ of 54.19 h. This finding differs significantly from a previous study in crucian carp (*Carassius auratus*), where the peak ABZ-SO concentration in skin-muscle reached only 0.787 $\mu\text{g/g}$ at 96 h after ABZ was administered orally at 12 mg/kg BW for 5 days [34]. In contrast, the reported $t_{1/2\lambda_z}$ in crucian carp was 47.28 h. These data suggest that Yellow River carp absorb ABZ more rapidly and excrete ABZ-SO more slowly compared to crucian carp.

Several factors may account for these differences. First, the cumulative dose was higher in the current study (7 days vs. 5 days), potentially leading to greater drug accumulation in tissues. Second, temperature is known to significantly influence drug metabolism in fish. It has been reported that for every 1 °C increase in temperature within the optimal range, the metabolic and excretory rates of fish can increase by approximately 10% [35]. In the crucian carp study, water temperature was maintained at 20 ± 1 °C, lower than the 23 ± 0.6 °C in the present study, which likely accelerated drug absorption and metabolism in Yellow River carp. Furthermore, the observed discrepancies may be attributed to interspecies variations in body weight, metabolic capacity, and gastrointestinal physiology (e.g., intestinal pH profiles).

Water temperature is a critical factor influencing the pharmacokinetics of drugs in fish. In a previous study, crucian carp were orally administered ABZ at 10 mg/kg BW for three consecutive days at different water temperatures [36]. The results showed that the $t_{1/2\lambda_z}$ of ABZSO at 10 °C was significantly longer (16.34 h) than at 25 °C (6.72 h). Similarly, the $t_{1/2\lambda_z}$ of ABZSO₂ was 12.82 h at 10 °C and 6.56 h at 25 °C. These findings suggest that higher water temperatures accelerate the metabolism of benzimidazoles in fish.

In the present study, Yellow River carp were reared at 23 ± 0.6 °C, and following repeated oral administration of ABZ (12 mg/kg BW) for seven consecutive days, the $t_{1/2\lambda_z}$ of ABZSO was 54.19 h. By comparison, in crucian carp exposed to a slightly lower temperature of 20 ± 1 °C, the $t_{1/2\lambda_z}$ of ABZSO was 47.28 h after five days of ABZ administration at the same dosage [23]. Interestingly, the elimination rate did not increase proportionally with temperature, suggesting that factors beyond water temperature—such as interspecies physiological differences, variations in drug formulation, or dosing duration—may also influence the metabolic rate of ABZ and its metabolites in different fish species.

To the best of our knowledge, this is the first study to report ABZ and ABZSO concentrations in the liver and kidney of Yellow River carp following multiple oral administrations. ABZ reached peak concentrations of 1.09 $\mu\text{g/g}$ in the liver and 2.16 $\mu\text{g/g}$ in the kidney at 24 h post-administration. For ABZSO, the $C_{\max}$ in the liver was 4.51 $\mu\text{g/g}$ at 24 h, while in the kidney, $C_{\max}$ was 3.35 $\mu\text{g/g}$ at 48 h. The concentrations of ABZ and ABZSO in liver and kidney were significantly higher than those in plasma and skin-on muscle, consistent with previous findings following single-dose administration [31].

Bile samples were not collected in this study, so the potential role of biliary excretion could not be assessed. Further studies are warranted to determine whether bile represents a

major excretory route for ABZ in fish. Notably, trace amounts of ABZ were still detectable in all tissues at 120 h, and ABZSO remained detectable up to 240 h post-treatment, indicating widespread distribution and slow elimination of both compounds in Yellow River carp.

In veterinary drug residue elimination studies, the tissue with the highest residue concentration and longest elimination time is typically selected as the target tissue for calculating the withdrawal period [37]. However, in China, consumers generally do not consume fish offal, especially from freshwater species. Therefore, in this study, skin-on muscle was chosen as the target tissue for withdrawal period determination. According to China's national food safety standard for veterinary drug residues, the MRL for ABZ-2-NH₂-SO₂ is 100 µg/kg, which corresponds to a calculated withdrawal period of 0 days.

Nonetheless, residues of ABZ and its metabolite ABZSO in skin-on muscle could still pose risks to consumers and the environment. Thus, the withdrawal period was also estimated based on the European Union's MRL for ruminant-derived food products [20], which sets an MRL of 100 µg/kg for the sum of ABZ and its three metabolites used as the residue marker. This calculation yielded a withdrawal period of 16 days.

It is important to note that drug residues in liver and kidney tissues should not be overlooked. Even after the withdrawal period, consumers should exercise caution during the processing of Yellow River carp by thoroughly removing all viscera to minimize the risk of exposure to residual veterinary drugs in internal organs.

Gut microbiota stability plays a crucial role in fish health, growth, and immune regulation [38]. It has been well established that drug treatments can alter the composition and structure of fish gut microbial communities [39]. However, most research to date has focused on the impacts of antibiotics [40,41] or feed additives [42,43] on fish gut microbiota, with comparatively fewer studies examining the effects of anthelmintics. In this study, we investigated the impact of ABZ, an anthelmintic approved for aquaculture use, on the intestinal microbiota of Yellow River carp (*Cyprinus carpio haematopterus*) at different dosing regimens using 16S rRNA high-throughput sequencing. Our results demonstrated that ABZ administration significantly reduced intestinal microbial diversity and richness in Yellow River carp, with both α - and β -diversity showing a dose-dependent decline. Notably, microbial diversity and abundance exhibited a trend toward recovery following drug withdrawal.

The results of this study showed that ABZ administration significantly affected the gut microbial community of Yellow River carp. At the phylum level, notable changes were observed in dominant groups such as *Pseudomonadota*, *Firmicutes*, and *Clostridia*. In particular, the relative abundance of the *Pseudomonadota* decreased progressively with prolonged exposure and higher dosages of ABZ, with only partial recovery observed following drug withdrawal. The *Pseudomonadota* phylum is taxonomically diverse and includes many genera of ecological and pathological significance in fish, such as *Vibrio*, *Aeromonas*, *Photobacterium*, *Edwardsiella*, *Acinetobacter*, and *Pseudomonas*.

The reduction in *Pseudomonadota* may reflect a broad-spectrum antimicrobial impact of ABZ, though the specific bacterial taxa affected remain to be clarified through deeper taxonomic and functional profiling. Given the inclusion of several major fish pathogens within this phylum, the observed microbial shifts could potentially influence host susceptibility to infection or disease progression. Future research is needed to determine whether ABZ exerts selective pressure against specific pathogens, such as *Aeromonas* or *Vibrio* spp., and to explore the implications for microbial resistance or dysbiosis.

This study provides a preliminary framework for understanding the interplay between antiparasitic drugs and host-associated microbial communities in aquaculture species. The potential of ABZ as part of integrated disease management strategies should be

approached cautiously, and its effects on both beneficial and pathogenic microbes warrant further investigation.

The observed reduction in the relative abundance of *Pseudomonadota* following ABZ feeding raises the question of whether ABZ can mitigate disease caused by pathogenic *Pseudomonas* species in fish, which warrants further investigation. This study also offers novel insights and potential avenues for combinatorial antimicrobial strategies targeting pseudomonad infections in aquaculture.

The phylum *Pseudomonadota* is a large and diverse group of bacteria, most of which are Gram-negative, possess cell walls, and commonly appear as rods or cocci. In contrast, members of the phylum *Bacteroidota* are anaerobic Gram-negative bacteria that normally inhabit the intestinal tracts and other body sites of animals. Both *Pseudomonadota* and *Bacteroidota* play important roles in energy metabolism within the host organism [44]. For example, bacteria from the *Pseudomonadota* phylum and species can produce polysaccharide-degrading enzymes that promote nutrient absorption in fish [45]. A previous study demonstrated that *Pseudomonadota* and *Mycobacterium* species metabolize carbohydrates to produce short-chain fatty acids (SCFAs)—including acetic acid, propionic acid, and butyric acid—which facilitate nutrient uptake by the host [46]. In this study, the relative abundance of Firmicutes and *Mycobacterium* increased with longer duration and higher doses of ABZ administration. These findings indicate that ABZ treatment in Yellow River carp does not negatively affect nutrient absorption and may even enhance it to some extent.

At the genus level, the relative abundance of *Streptococcus digestans* and *Aeromonas* spp. increased with higher doses and longer durations of ABZ administration and did not exhibit a recovery trend following drug withdrawal. This suggests that ABZ treatment disrupted the structural composition of the intestinal microbiota in Yellow River carp. *Streptococcus* spp. are facultative anaerobic bacteria commonly found in the intestines of animals, and certain species are recognized as major pathogens in both humans and fish. Notably, *Streptococcus iniae*, *S. parauberis*, and *S. dysgalactiae* are among the most important pathogenic species in aquaculture, causing severe infections in a variety of fish species [47]. The persistent increase in their relative abundance may suggest a potential risk for disease outbreaks following prolonged ABZ exposure, highlighting the need for careful assessment of drug impacts on gut microbial ecology in aquaculture settings. *Aeromonas* spp., also facultative anaerobes, are ubiquitous in freshwater environments, soil, and other habitats. They are recognized as major pathogens in fish and other poikilothermic animals [48]. For instance, *Aeromonas hydrophila* and other virulent *Aeromonas* strains have been implicated in severe disease outbreaks in aquaculture, often resulting in high mortality rates and significant economic losses [49]. Although ABZ is effective in eliminating parasitic infections in fish, it may inadvertently increase the abundance of potentially pathogenic genera such as *Aeromonas* in the gut microbiota. Therefore, it is important to closely monitor fish health following ABZ treatment.

5. Conclusions

Following oral administration of ABZ at a dose of 12 mg/kg BW for seven consecutive days at 23 ± 0.6 °C, ABZ and its primary metabolite ABZSO were widely distributed in Yellow River carp and eliminated slowly. In contrast, ABZSO₂ and ABZ-2-NH₂-SO₂ were detected only in trace amounts. The liver and kidneys were the main metabolic organs. According to China's MRL (100 µg/kg for ABZ-2-NH₂-SO₂), the withdrawal period is zero days. However, adopting the EU's MRL (100 µg/g for the total residues of four compounds), a withdrawal period of 16 days (351 °C-days) is required.

ABZ administration also significantly altered the gut microbiota, reducing OTU richness and alpha diversity, and shifting community composition. The relative abun-

dance of *Pseudomonadota* decreased, while *Firmicutes*, *Actinomycetota*, *Aeromonas* spp., and *g_Peptostreptococcaceae_Incertae_Sedis* increased, with limited recovery after drug withdrawal. These findings provide critical data for the rational use of ABZ in aquaculture and highlight its potential ecological impacts.

Author Contributions: F.Y. (Fan Yang) and X.-P.L. conceived and designed the experiments. Y.L., Y.D. and Y.-N.Z. conducted the animal experiments, performed data analysis, and drafted the manuscript. W.-R.W., Y.-X.C. and Y.-G.J. carried out the gut microbial community analysis. L.-J.S., S.-H.L. and F.Y. (Fang Yang) were responsible for determining the concentrations of ABZ and its metabolites in fish samples. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: For this experiment, ethical approval was obtained from the Institutional Animal Care and Use Committee (IACUC) of Henan University of Science and Technology (Approval No. 20240302) for handling Yellow River carp.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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