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# Pharmacokinetics, Pharmacodynamics and Depletion of Florfenicol Applied in White Leg Shrimp (*Litopenaeus vannamei*) Aquaculture and Impact on Shrimp Hepatopancreas Histology

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## Abstract

Florfenicol (FF) is one of the common antimicrobials used to control bacterial disease in shrimp aquaculture. This study aimed to determine the pharmacokinetics (PK) parameters of FF in white leg shrimp plasma, hepatopancreas and muscle as well as its residue depletion in shrimp muscle and the impact on shrimp hepatopancreas histology during and after FF medication. In the PK experiment, shrimp were fed once at 10 mg FF/kg body weight (bw) via oral in-feed administration to determine PK parameters in plasma, hepatopancreas and muscle. The maximum concentration ($C_{max}$) of 60.56 µg/L in plasma was observed after 1.77 h ($T_{max}$). In muscle, a $C_{max}$ of 11.76 µg/kg was attained after 0.20 h, while in hepatopancreas, the $C_{max}$ was higher (386.92 µg/kg) and was rapidly obtained ($T_{max}$ = 0.19 h). The $C_{max}$ values in shrimp plasma were below the minimum inhibitory concentration (MIC) against *Vibrio parahaemolyticus*, known to cause acute hepatopancreatic necrosis disease (AHPND) in shrimp. Therefore, it can be concluded that to ensure the effectiveness of this treatment, the dose should be higher than 10 mg FF/kg bw. FF depletion in white leg shrimp muscle and its histological impact on hepatopancreas were determined after feeding FF-medicated feed once-a-day or twice-a-day for 3 consecutive days with a dose of 10 mg FF/kg bw. The residues in shrimp muscle were rapidly eliminated and fell below the limit of quantification at 24 h after stopping medication. The withdrawal time of FF in shrimp muscle was 27.9 degree-days (2 days at 26.5 °C) according to the maximum residue limit (MRL) of 100 µg/kg set by the European Commission and Korean Ministry and when feeding FF twice-a-day for 3 days. The results from histological analysis showed that there was no negative effect on shrimp hepatopancreas after stopping medication in both once- and twice-a-day treatments.

**Keywords:** depletion; florfenicol; hepatopancreas; pharmacokinetics; white leg shrimp

**Key Contribution:** This study clarifies how florfenicol is distributed in white leg shrimp and its pharmacokinetic properties as well as the impact on hepatopancreas; and a suitable withdrawal time of florfenicol is defined ensuring the safety of shrimp products.



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## 1. Introduction

White leg shrimp (*Litopenaeus vannamei*) is being cultured in Vietnam with an annual production of 1.12 million tons in 2023 [1]. Since the development of intensive and super-intensive farming systems, the Vietnamese shrimp sector is facing issues such as disease outbreaks and increase in chemical use related to the increase in stocking density [2]. Shrimp diseases are mainly due to pathogens such as viruses and bacteria, causing, e.g., acute hepatopancreatic necrosis disease (AHPND), white feces disease and white spot syndrome [3–5]. In particular, diseases caused by *Vibrio* spp. result in serious economic losses [6,7]. Several researchers [8–10] have reported that shrimp farmers normally use antibiotics to manage shrimp health as well as prevent and treat bacterial infections.

Florfenicol (FF) is one of the antibiotics commonly used for the treatment of bacterial diseases in white leg shrimp and other aquaculture species [9,11–13]. FF is a broad-spectrum synthetic bacteriostatic antibiotic belonging to the phenicol group. The mode of action of FF is similar to that of chloramphenicol, acting through the inhibition of protein synthesis via binding to the S50 ribosomal subunit of the target microorganism, resulting in the prevention of protein chain elongation in bacterial cells, and the inhibition of bacterial growth [14–16]. Since chloramphenicol is prohibited for use in aquaculture, FF is used as an alternative because FF does not have the potential side-effects of chloramphenicol [17]. FF is one of the antibiotics approved by the US Food and Drug Administration for aquaculture that has been shown to be effective in treating diseases caused by Gram-positive and Gram-negative bacteria in aquaculture and livestock [18–20].

In aquaculture, FF pharmacokinetics (PK) have mostly been studied in fish, e.g., catfish [21–24], Atlantic salmon [25,26], lumpfish [27], orange-spotted grouper [28], cod [29], crucian car [30], olive flounder [31], rainbow trout [32,33], Asian tilapia [34] and seabass [35]. While several PK studies have been published on fish, such studies on shrimp are limited. For instance, FF PK studies on white leg shrimp were investigated by Fang et al. [36] and Ren et al. [11] in China, and in black tiger shrimp by Tipmongkolsilp et al. [37] in Thailand. These studies found that the values of $C_{max}$ (μg/g) and $T_{max}$ (h) in the hepatopancreas of shrimp treated with FF by oral administration were as follows: white leg shrimp 164 and 0.5 (at a dose of 10 mg FF/kg bw), black tiger shrimp 0.7 and 1 (at 0.8 g FF/kg feed), respectively [36,37]. However, PK parameters vary among shrimp species living in different locations or environments [11,36]. For instance, after oral administration at a dose of 30 mg/kg bw, the $T_{max}$ value of FF in the plasma of shrimp (*Exopalaemon carinicauda*) at 28 °C ($T_{max}$ of 0.5 h) was faster than that at 22 °C ($T_{max}$ of 1 h); the corresponding values for shrimp hepatopancreas were 1 h and 2 h, respectively [38]. In addition, PK parameters within a single fish species may differ due to factors like geographic origin and genetic variation caused by selective breeding and artificial selection [39]. To determine an effective therapeutic dose as well as interval time when using FF in shrimp aquaculture to be effective in treating bacterial infections, PK parameters are essential to establish and to compare with a pharmacodynamic (PD) parameter, namely, the minimum inhibitory concentration (MIC) against the bacteria causing the disease, which is called the pharmacokinetics/pharmacodynamics (PK/PD) index. This may help to reduce antibiotic resistance of pathogens in shrimp farming.

Hepatopancreas (also called the midgut gland) health is one of the indicators which may be used to predict the health status of shrimp [40]. The hepatopancreas of shrimp and other crustaceans is the most sensitive organ to drug and chemical exposure. Several studies have revealed that the improper use of antibiotics or chemicals has caused serious adverse effects on shrimp health, especially on sensitive organs like the hepatopancreas. The higher the dose of antibiotics used, the more severe is the negative impact on shrimp hepatopancreas [41–44]. This evidence of negative effects on the hepatopancreas after

extensive duration of treatments with excessive doses should be a strong caution to shrimp farmers who have overused antibiotics or chemicals in shrimp aquaculture. Although FF is commonly used in shrimp culture, there has been no study on the side-effects of FF on white leg shrimp hepatopancreas.

Based on the above evidence, research on the PK, PD and withdrawal time of FF in shrimp should be conducted to evaluate how FF is processed by shrimp, as well as to study the impact of using FF medicated feed on the shrimp hepatopancreas to monitor shrimp health and to ensure the safety of shrimp food products.

## 2. Materials and Methods

### 2.1. Chemicals and Equipment

High purity chemicals (>98%) meeting the FF standard and internal standard (penicillin V) were provided by Dr. Ehrenstorfer® (Augsburg, Germany). The instrument used for FF quantification was a Waters ACQUITY Ultra-High-Performance Liquid Chromatograph (UHPLC) (Waters, Milford, MA, USA) with an Acquity UPLC CSH C18 column, 2.1 mm × 50 mm, 1.7 µm (Waters, Milford, MA, USA). C18 Bondesil powder with a particle size of 40 µm was obtained from Agilent (Santa Clara, CA, USA). Other chemicals, including acetonitrile, methanol and distilled water, were purchased from Merck (Darmstadt, Germany).

### 2.2. Bacterial Strains

A total of 42 bacterial strains were used for antimicrobial tests. These strains were isolated from AHPND shrimps which were collected from shrimp farms located in 7 provinces of the Mekong Delta, including Ca Mau, Kien Giang, Soc Trang, Can Tho, Dong Thap, Tra Vinh and Tien Giang from 2022 and 2024. Strains were identified as *Vibrio parahaemolyticus* (*V. parahaemolyticus)* and stored at −80 °C in tryptone soy broth (TSB, Merck) containing 25% glycerol and supplemented with 1.5% (*w*/*v*) sodium chloride.

#### 2.2.1. Antibiotic Susceptibility Testing

*V. parahaemolyticus* strains from glycerol stock were recovered by inoculating into tryptic soy agar supplemented with 1.5% NaCl ($TSA^+$) and incubating at 28 °C for 18 h. The color and shape of colonies were recorded. Gram stain was applied to check the purity of the bacteria. Pure bacterial colonies were cultured to increase the biomass in nutrient broth at 28 °C for 24 h and the concentration of bacteria was adjusted to an approximate density of $10^6$ CFU/mL using McFarland's 0.5 Barium Sulfate Standard Solution [45].

The resistance of *V. parahaemolyticus* to FF was determined by an agar disk diffusion method as described by Balouiri et al. [46] and Jiang et al. [47], with some modifications. Disks containing 30 micrograms of FF were used. One hundred microliters of bacterial suspension were evenly spread over the surface of the $TSA^+$ plate. After 15 min, an FF disk was gently fixed onto the agar surface using a sterile fine-pointed forceps to ensure complete contact and the plate was incubated at 28 °C for 24 h. The diameters of the inhibition zones (as judged by the unaided eye), including the diameter of the disk, were measured and the size of the clear zone of inhibition was used to classify isolates as susceptible, intermediate, or resistant according to the guideline of the Clinical and Laboratory Standards Institute (CLSI) [48].

#### 2.2.2. Determination of the Minimum Inhibitory Concentration (MIC) of FF

The bacteria were activated as described above and the bacterial initial concentration was $10^6$ CFU/mL. The MIC of FF was determined for each *V. parahaemolyticus* strain using the broth macro-dilution method with some modifications [49]. An FF standard solution

with a final concentration of 1280 μg/100 μL was prepared using sterile distilled water. Serial dilutions were then prepared to obtain 12 decreasing concentrations. The bacterial suspension (1 mL) was 200-fold diluted with $TSB^+$ medium. Fourteen sterilized screwed capped test tubes were prepared and labeled, which were then loaded with 4.9 mL of diluted bacterial suspension. After that, 100 μL of the prepared FF standard solutions was added to achieve a final concentration of 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, and 0.125 μg/mL. Simultaneously, one positive control tube ($TSB^+$ solution with bacteria) and one negative control tube ($TSB^+$ solution without bacteria) were also prepared. All these test tubes were incubated for 24 h at 28 °C. MIC was defined as the lowest concentration of FF that showed no visible bacterial growth.

### 2.3. Shrimp

Healthy white leg shrimp (*Litopenaeus vannamei*), weighing 10–15 g each, were obtained from the Faculty of Marine Science and Technology, College of Aquaculture and Fisheries, Can Tho University. The shrimp had been reared from the post-larval stage without the use of antibiotics prior to the start of the experiment. They were stocked in 2 $m^3$ tanks at a density of 100 shrimp/$m^3$ and acclimated for one week.

During the acclimation period, shrimp were fed a commercial pelleted feed (Proconco, Can Tho City, Vietnam) containing 40% crude protein, administered four times daily (06:00, 10:00, 14:00, and 18:00 h) at a feeding rate equivalent to 3% of the shrimp's body weight. Uneaten feed, which was consistently less than 5% of the total offered, was removed 30 min post-feeding via siphoning.

Experimental tanks were equipped with a recirculating water system and aeration to maintain dissolved oxygen levels above 5 mg/L. Key water quality parameters were regularly monitored and maintained within optimal ranges for shrimp culture: salinity was adjusted to 10‰, alkalinity was maintained at 140–160 mg $HCO_3^-$/L, and temperature, pH, and nitrite levels were also closely controlled. All these environmental parameters were monitored throughout the study.

Vime-fenfish 2000, a product containing 20% florfenicol (FF), was sourced from the Vemedim Company (Can Tho City, Vietnam). Prior to the experiment, the absence of FF residues in shrimp tissues and the actual concentration of FF in the product were verified using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS).

### 2.4. Pharmacokinetics Experiment

#### 2.4.1. Experimental Design

The experimental procedure followed the protocol described by Huynh et al. [50]. White leg shrimp were distributed and acclimated in six separate tanks, corresponding to six biological replicates. Each group received a single oral dose of florfenicol (FF) at 10 mg/kg body weight (bw). This dosage was chosen based on standard therapeutic recommendations provided by commercial product manufacturers. Additionally, a national survey on antibiotic usage in Vietnamese shrimp farming reported the same dosage [12]. Previous studies have also employed this dose effectively to manage bacterial infections in aquatic animals [24,36].

The required FF concentration in the feed was calculated based on the estimated feed intake for 1 kg of shrimp biomass. To prepare the medicated feed, FF 20% (Vime-fenfish 2000) was manually mixed with commercial pellets to achieve a final concentration of 1333 mg FF/kg feed. The antibiotic and feed were thoroughly blended in a cylindrical container for 5 min. Subsequently, the mixture was coated with 2% fish oil and air-dried for 15 min before administration at 06:00 h. For the remaining daily feedings (10:00, 14:00, and 18:00 h), shrimp received non-medicated feed.

#### 2.4.2. Sample Collection

Sampling was conducted at 0.5, 1, 2, 4, 8, 12, and 24 h post-treatment. At each time point, three shrimp were collected from one tank (one replicate). Approximately 0.5 mL of blood was drawn from the pericardial cavity of each shrimp using a 1 mL syringe pre-filled with an anticoagulant solution (0.01 M EDTA, 0.338 M NaCl, 0.115 M glucose, and 0.03 M trisodium citrate dihydrate). Blood samples from three shrimp within a tank were pooled into a 1.5 mL centrifuge tube and centrifuged at 9500× *g* for 5 min to separate the plasma.

Simultaneously, hepatopancreas and muscle tissues were excised from the same three shrimp and pooled into labeled plastic bags to constitute one replicate. All collected plasma, muscle, and hepatopancreas samples were immediately frozen and stored at −80 °C until further analysis.

### *2.5. Depletion of Florfenicol in Muscle Tissue and Hepatopancreas Histological Observation*

#### 2.5.1. Depletion of Florfenicol in Shrimp Muscle

FF depletion in white leg shrimp was assessed following oral administration. The study was conducted using two treatment groups, each consisting of three replicate tanks. Shrimp in Group 1 received FF-medicated feed (prepared as previously described) once daily at 06:00 h, whereas shrimp in Group 2 were fed the same medicated feed twice daily at 06:00 and 18:00 h. The treatment lasted for three consecutive days at a dosage of 10 mg FF/kg body weight.

Following the treatment period, all shrimp were fed non-medicated feed for 21 days, administered four times daily at 06:00, 10:00, 14:00, and 18:00 h. Muscle samples were collected from shrimp on days 1 and 3, one hour after administration of the medicated feed. Subsequent samplings were conducted on days 1, 3, 7, 14, and 21 post-treatment. At each time point, ten shrimp were randomly collected from each tank. Muscle tissues were excised, homogenized, pooled, and stored at −80 °C until extraction and analysis.

#### 2.5.2. Shrimp Hepatopancreas Histological Examination

In addition to FF residue analysis, hepatopancreas samples were collected for histological examination to assess potential impacts of FF exposure. For this purpose, three shrimp per tank were sampled at three time points: one day prior to FF administration (baseline), one hour after the final FF dose, and seven days after treatment cessation. Hepatopancreatic tissues were preserved and processed into histological sections following the procedure described by Huynh et al. [50]. Histological examinations were conducted using a Novex microscope (Arnhem, the Netherlands). The numbers of B- and F-cells were counted according to the modified method of Nima et al. [51]. Cell counts were performed within a consistent microscopic field, randomly selecting different fields across 40 tubules per shrimp.

Statistical analyses of dosing and/or sampling effects were conducted using one-way and two-way ANOVA with SPSS 20.0 software. Duncan's multiple range test was employed for post hoc comparisons. Differences were considered statistically significant at $p < 0.05$.

### *2.6. Florfenicol and Florfenicol Amine Quantification*

FF and florfenicol amine (FFA) in shrimp plasma were extracted and analyzed using a modified version of the procedure described by Pham et al. [24]. In brief, 0.5 mL of plasma was spiked with penicillin V solution as an internal standard (IS) and mixed with 3 mL of acetonitrile. The mixture was then centrifuged at 9503× *g* for 10 min, the supernatant was collected, dried under nitrogen, and the dry residue was dissolved in the mobile phase. Before LC–MS/MS injection, the extract was filtered through a 0.22 µm nylon membrane filter (Advantec MFS, Dublin, CA, USA).

Hepatopancreas and muscle samples were separately homogenized using an Ultra Turrax homogenizer (T-25, IKA, China). The extraction method was adapted from the United States Department of Agriculture (USDA) [52] with some modifications. Briefly, 3.00 ± 0.1 g of homogenized sample was transferred into a 50 mL tube, spiked with 60 µL of 1 µg/mL IS, vortexed, and incubated at room temperature for 15 min. After incubation, 1 mL of 0.1 M phosphate buffer (pH 8.0) was added to the tube, which was vortexed for 1 min, followed by the addition of 20 mL of acetonitrile. The mixture was vortexed for 30 sec and sonicated for 15 min in an Elma Ultrasonic bath (Elma Hans Schmidbauer, Singen, Germany). Samples were then centrifuged (22R, Andreas Hettich, Tuttlinggen, Germany) at 3501× *g* for 5 min to collect the supernatant. Acetonitrile was added to adjust the final volume to 24 mL, then 8 mL was transferred to a 15 mL glass test tube containing 40 mg Bondesil C18 and vortexed for 20 min. After centrifugation at 3501× *g* for 4 min, the supernatant was collected into a 10 mL glass test tube and then evaporated at 40 ± 1 °C in a water bath under nitrogen flow. The dried residues were reconstituted in 1 mL of mobile phase [acetonitrile and methanol (1:1) with 0.1% formic acid in water], filtered through a 0.22 µm nylon membrane filter (Advantec MFS, Dublin, CA, USA) and injected into the LC–MS/MS system.

Detection and quantification of FFA and FF were performed using a Xevo TQ-S triple quadrupole mass spectrometer (Waters) equipped with an electrospray ionization (ESI) probe. The ESI probe operated in positive ion mode for FFA detection and in negative ion mode for FF. Optimization of the mass spectrometry parameters included capillary voltage, ion source temperature, cone gas flow, desolvation gas flow, and desolvation temperature. Argon was used as the collision gas, and the collision cell pressure was $4 \times 10^{-3}$ mBar. For quantitation (shown in bold) and confirmation, the following two specific fragmentation transitions (precursor > product ion, $m/z$) were used, with the collision energy shown in brackets: FF **355.97 > 184.94** (24 eV) and 355.97 > 335.99 (14 eV); FFA **248.07 > 130.21** (24 eV) and 248.07 > 91.07 (44 eV); penicillin V (IS) **351.04 > 229.09** (18 eV) and 351.04.1 > 257.04 (14 eV).

The limits of detection (LOD) were 2.5 µg/kg for FF and 5 µg/kg for FFA in all matrices, while the limits of quantification (LOQ) were 5 and 10 µg/kg, respectively. Both LOD and LOQ were determined following AOAC guidelines [53]. The working ranges of the standard calibration curve, prepared using blank samples, exhibited a linear range of 5–80 µg/kg for FF and 10–80 µg/kg for FFA. Extraction recovery ranged from 92% to 99%. Linearity, specificity, precision (repeatability and within-laboratory reproducibility), apparent recovery, decision limit ($CC_\alpha$) and detection capability ($CC_\beta$) were evaluated and validated according to Commission Decision 2002/657/EC [54]. All parameters fell within the acceptance criteria.

### *2.7. Pharmacokinetics Data Analysis*

PK modeling of FF concentrations in plasma, hepatopancreas and muscle tissues was performed using a naïve pooled population approach, applying a one-compartmental model with first-order absorption and elimination. In plasma, the following PK parameters were calculated: absorption rate constant ($k_a$), absorption half-life ($T_{1/2abs}$), maximal plasma concentration ($C_{max}$), time to maximal plasma concentration ($T_{max}$), area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-inf}$), elimination rate constant ($k_{el}$), elimination half-life ($T_{1/2el}$), apparent total body clearance after oral administration (Cl) and apparent volume of distribution after oral administration ($V_d$).

For hepatopancreas and muscle tissues, the calculated parameters included the maximal tissue concentration ($C_{max}$), time to maximal tissue concentration ($T_{max}$), and area under the tissue concentration–time curve from time 0 to infinity ($AUC_{0–inf}$), which was

computed using the linear-up log-down trapezoidal method. All data analysis and PK modeling were conducted using Phoenix 8 (Certara, Princeton, NJ, USA).

### *2.8. Withdrawal Times Calculation*

To estimate the withdrawal time (WT) of FF, the WT was determined using the WT 1.4 software, developed by Hekman [55], in accordance with the guidelines issued by the European Medicines Agency (EMA) [56]. The FF residue concentrations in shrimp muscle tissue were measured at multiple post-treatment time points. These concentrations were analyzed using linear regression against time expressed in degree-days, which were calculated by multiplying the average daily water temperature (°C) by the total number of days the temperature was measured. The WT was defined as the time point at which the upper one-sided 95% tolerance limit for FF residues dropped below the maximum residue limit (MRL) of 100 μg/kg—established by both the EU and Korea—with 95% confidence.

## 3. Results

### *3.1. Antimicrobial Activity*

Disk diffusion tests. The antimicrobial activity of FF towards 42 *V. parahaemolyticus* strains, evaluated by the disk diffusion method (30 μg FF disk), expressed as inhibition zone diameters, were 6 to 36 mm for strains collected in Ca Mau, 22 to 26 mm for strains coming from Kien Giang, 18–36 mm (Soc Trang), 24–25 mm (Can Tho), 30 mm (Dong Thap), 25–28 mm (Tra Vinh), 25–28 mm (Tien Giang) (Table 1).

**Table 1.** The MIC (μg/mL) and inhibition zone diameters (mm) of FF for *V. parahaemolyticus* strains.

| Isolate Number | Inhibition Zone Diameter | MIC | Isolate Number | Inhibition Zone Diameter | MIC |
|---|---|---|---|---|---|
| 1 | 36 | 8 | 22 | 34 | 8 |
| 2 | 22 | 1 | 23 | 24 | 4 |
| 3 | 21 | 1 | 24 | 26 | 4 |
| 4 | 24 | 1 | 25 | 22 | 1 |
| 5 | 28 | 2 | 26 | 21 | 8 |
| 6 | 30 | 2 | 27 | 18 | 256 |
| 7 | 31 | 8 | 28 | 24 | 8 |
| 8 | 6 | >256 | 29 | 23 | 16 |
| 9 | 22 | 4 | 30 | 22 | 4 |
| 10 | 24 | 2 | 31 | 25 | 8 |
| 11 | 20 | 4 | 32 | 25 | 4 |
| 12 | 30 | 4 | 33 | 24 | 4 |
| 13 | 18 | 256 | 34 | 30 | 4 |
| 14 | 24 | 8 | 35 | 25 | 4 |
| 15 | 31 | 16 | 36 | 25 | 4 |
| 16 | 18 | 256 | 37 | 26 | 4 |
| 17 | 25 | 16 | 38 | 28 | 2 |
| 18 | 26 | 4 | 39 | 27 | 8 |
| 19 | 22 | 2 | 40 | 27 | 8 |
| 20 | 36 | 4 | 41 | 25 | 8 |
| 21 | 31 | 8 | 42 | 28 | 8 |

Isolates numbered 1 to 17 collected in Ca Mau province, 18–19 from Kien Giang province, 20–30 from Soc Trang province, 31–33 from Can Tho University, 34 from Dong Thap province, 35–39 from Tra Vinh province, 40–42 from Tien Giang province.

Minimum inhibitory concentration. Figure 1 shows the repartition of MIC values of FF for 42 *V. parahaemolyticus* strains isolated from 7 provinces of the Mekong Delta, Vietnam. For thirty-five strains of *V. parahaemolyticus* (83.3%), the FF MIC ranged between 1 and 8 μg/mL. For approximately 7% (3/42 strains) of the *V. parahaemolyticus* tested strains, the

FF MIC was 16 µg/mL and for four strains (about 10%), FF MIC was above 256 µg/mL (Figure 1). In Ca Mau province (isolates 1–17), 5/18 isolates had MIC values ranging above 8 µg/mL while 2/10 isolates from Soc Trang province had (Table 1).

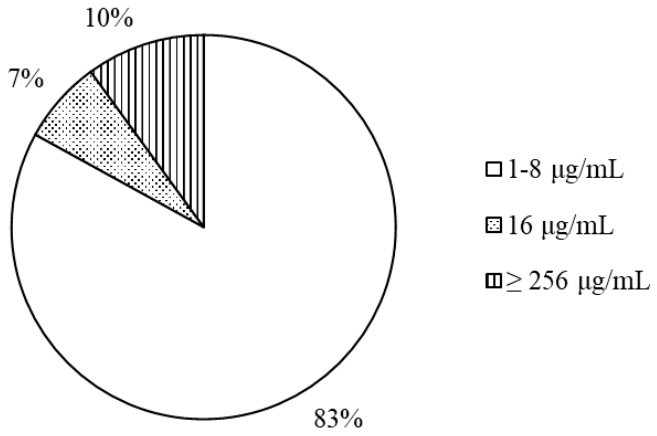


**Figure 1.** The repartition (%) of FF MIC (µg/mL) for 42 strains of *V. parahaemolyticus* collected in 7 provinces of the Mekong Delta, Vietnam.

### *3.2. Pharmacokinetics of Florfenicol in Plasma, Hepatopancreas and Muscle of White Leg Shrimp*

After administering a single 10 mg/kg body weight dose of FF, the concentration–time curves indicated that, at 30 min after FF ingestion, the highest FF concentrations were observed in the hepatopancreas of white leg shrimp, compared to lower levels in plasma and muscle (Figure 2).

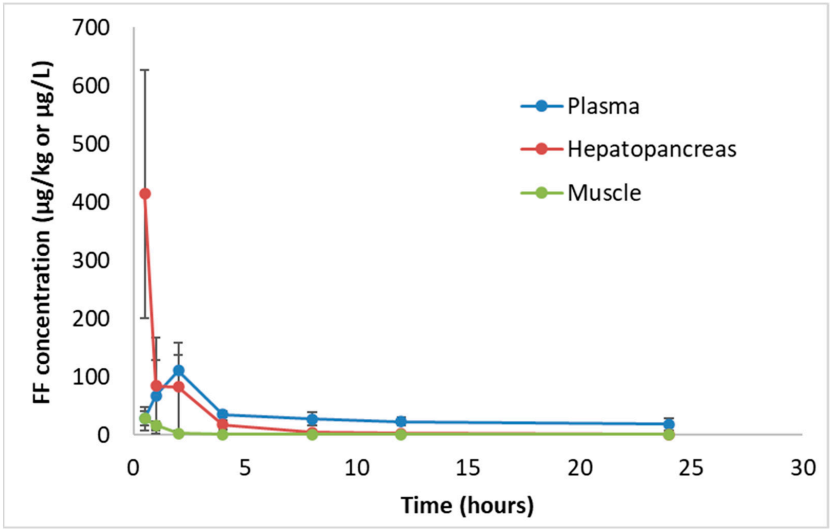


**Figure 2.** FF concentrations (mean ± SD, *n* = 6, µg/L or µg/kg, wet basis) in the plasma, hepatopancreas and muscle of white leg shrimp after a single oral administration of 10 mg/kg bw.

The estimated PK parameters of FF in white leg shrimp plasma were determined by a one-compartment model. The results are shown in Table 2. The absorption rate constant ($k_a$) was 0.066 $h^{-1}$, the absorption half-life ($T_{1/2abs}$) was 10.44 h, the maximal plasma concentration ($C_{max}$) was 60.56 µg/L, the time to reach the maximal plasma concentration ($T_{max}$) was 1.77 h, the apparent total body clearance after oral administration (Cl) was 9.75 L/kg/h, the apparent volume of distribution after oral administration ($V_d$) was 4.93 L/kg, the area under the plasma concentration–time curve from time 0 to infinity ($AUC_{0–inf}$) was 1026.07 µg·h/L, the elimination rate constant ($k_{el}$) was 1.97 $h^{-1}$ and the elimination half-life ($T_{1/2el}$) was 0.35 h.

**Table 2.** Values for the main PK parameters of FF in white leg shrimp plasma after oral administration of a single dose of 10 mg FF/kg bw.

| PK Parameter | Unit | Plasma Estimate | CV% |
|---|---|---|---|
| $k_a$ | $h^{-1}$ | 0.066 | 13.88 |
| $T_{1/2abs}$ | h | 10.44 | 13.88 |
| $C_{max}$ | μg/L | 60.56 | 7.45 |
| $T_{max}$ | h | 1.77 | 10.41 |
| Cl | L/kg/h | 9.75 | 13.85 |
| $V_d$ | L/kg | 4.93 | 30.51 |
| $AUC_{0-inf}$ | μg·h/L | 1026.07 | 13.85 |
| $k_{el}$ | $h^{-1}$ | 1.97 | 17.96 |
| $T_{1/2el}$ | h | 0.35 | 17.96 |

Values are presented as the mean of the population estimate, $n$ = 6. Absorption rate constant ($k_a$), absorption half-life ($T_{1/2abs}$), maximal plasma concentration ($C_{max}$), time to maximal plasma concentration ($T_{max}$), area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-inf}$), elimination rate constant ($k_{el}$), elimination half-life ($T_{1/2el}$), apparent total body clearance after oral administration (Cl) and apparent volume of distribution after oral administration ($V_d$).

Regarding the estimated PK parameters of FF in white leg shrimp tissues, the results indicated a markedly higher concentration in the hepatopancreas compared to muscle (Table 3). The maximum concentration of FF in the hepatopancreas reached 386.92 μg/kg, while $C_{max}$ in shrimp muscle was significantly lower at 11.76 μg/kg. Similarly, based on $AUC_{0-inf}$ values, the distribution of FF in the hepatopancreas (1660 μg·h/kg) was much higher than that in shrimp muscle (88.0 μg·h/kg). In addition, $T_{max}$ values for these two organs were similar, approximately 0.2 h. However, the $T_{max}$ value of the hepatopancreas could not be accurately estimated because of the high standard deviation of FF concentrations in this organ (Figure 2).

**Table 3.** Values for the main PK parameters of FF in white leg shrimp hepatopancreas and muscle tissues after oral administration of a single dose of 10 mg FF/kg bw.

| PK Parameter | Unit | Hepatopancreas Estimate | Muscle Estimate |
|---|---|---|---|
| $C_{max}$ | μg/kg | 386.92 | 11.76 |
| $T_{max}$ | h | 0.19 | 0.20 |
| $AUC_{0-inf}$ | μg·h/kg | 1660 | 88.0 |

Values are presented as the mean of the population estimate, $n$ = 6. Maximal tissue concentration ($C_{max}$), time to maximal tissue concentration ($T_{max}$) and area under the tissue concentration-time curve from time 0 to inf ($AUC_{0-inf}$).

Florfenicol amine, a metabolite of FF, was not detected in the plasma, muscle or hepatopancreas of shrimp.

### *3.3. Depletion and Withdrawal Time of Florfenicol in White Leg Shrimp Muscle*

The residual levels of FF in white leg shrimp muscle of both once-a-day and twice-a-day treatments were very low. The mean FF concentrations in shrimp muscle were 3.65 μg/kg for once-a-day treatment and 20.53 μg/kg for twice-a-day treatment on the first day of medication. After that, the FF levels of shrimp muscle increased and reached the highest mean concentration on day 3 of medication, at 9.35 and 49.29 μg/kg in the once-a-day and twice-a-day treatments, respectively. FF concentrations in shrimp muscle were eliminated very quickly; the FF residual level fell below the LOQ (5 μg/kg) at 24 h post-medication and was lower than the LOD (2.5 μg/kg) from day 3 after stopping medication in both treatments of feeding shrimp with medicated feed once-a-day and twice-a-day (Table 4). Florfenicol amine was not detected in all samples (LOD and LOQ of 5 μg/kg and 10 μg/kg for FFA, respectively).

**Table 4.** Residual levels of FF in white leg shrimp muscle during and after medication at a dose of 10 mg FF/kg bw fed once-a-day and twice-a-day during 3 consecutive days. Values are presented as mean ± SD, $n$ = 3.

| Period | Sampling Time (Days) | FF Level (Once-a-Day Treatment) (µg/kg) | FF Level (Twice-a-Day Treatment) (µg/kg) |
|---|---|---|---|
| Medication | 1 | 3.65 ± 0.16 | 20.53 ± 4.52 |
| | 3 | 9.35 ± 3.10 | 49.29 ± 10.1 |
| After stopping medication | 1 | 3.03 ± 1.61 | 2.95 ± 0.95 |
| | 3 | <LOD | <LOD |
| | 7 | <LOD | <LOD |
| | 14 | <LOD | <LOD |
| | 21 | <LOD | <LOD |

After three consecutive days of medication at a dose of 10 mg FF/kg bw once-a-day, and an ambient temperature of 26.5 °C, the FF residues in shrimp muscle were lower than the MRL of 100 µg/kg set by EU and Korea. The WT estimated for FF only administered twice-a-day, was 27.9 degree-days (2 days at 26.5 °C) (Figure 3).

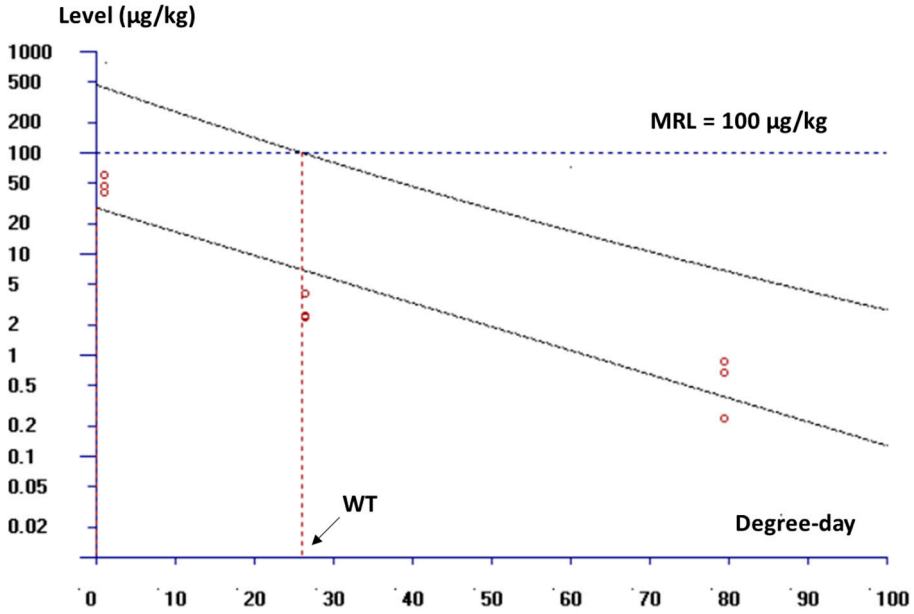


**Figure 3.** Plot of residual levels of florfenicol in white leg shrimp muscle recorded at 1 h, 24 h and 72 h, corresponding to 1.1, 26.5 and 79.5 degree-days, respectively, following the cessation of treatment as a function of degree-days. Shrimp had been administered FF orally at a dose of 10 mg/kg bw, twice-a-day for three consecutive days, under ambient conditions (26.5 °C). The withdrawal time was calculated as the time when the upper limit of the one-sided 95% confidence interval was below the MRL of 100 µg/kg.

### *3.4. Hepatopancreas Histology of White Leg Shrimp*

Prior to medication, histological examination of the hepatopancreas revealed a normal tissue structure with a full complement of B- and F-cells (Figure 4A). No abnormal clinical signs, such as mortality, color changed or reduced feed intake, were observed during medication. After oral administration of medicated feed twice-a-day for three consecutive days, a reduction in B-cell numbers was observed on day 3 of medication (Figure 4B). In Figure 4C, the numbers of B-cells in shrimp hepatopancreas were recovered on seven days after stopping medication (see also Figure 5).

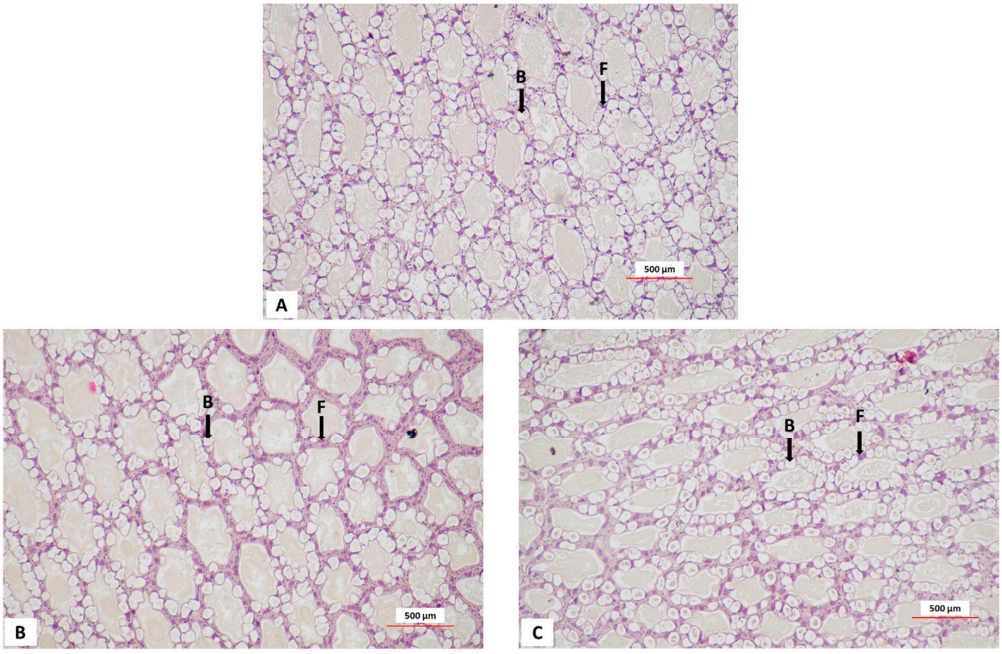


**Figure 4.** Representative histological section of shrimp hepatopancreas before (**A**) and after feeding with FF-medicated feed twice-a-day treatment on day 3 of medication (**B**) and 7 days after stopping medication (**C**) (100×). B—blasenzellen cells, F—fibrillenzellen cells.

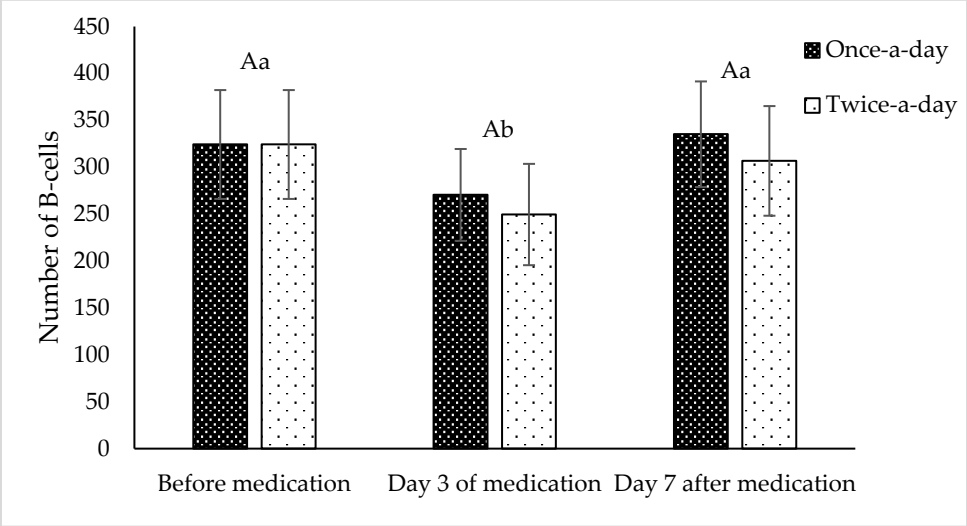


**Figure 5.** Number of B-cells of shrimp hepatopancreas before FF medication, on day 3 of medication and on day 7 after stopping medication in once-a-day (dark column) and twice-a-day (light column) treatments with 10 mg FF/kg bw. Values with similar capitalized letter showed no significant different between doses ($p > 0.05$). Values with similar small letter showed no significant different between sampling time ($p > 0.05$).

Results of the number of hepatopancreas B-cells after feeding shrimps with FF medicated feed once-a-day or twice-a-day for 3 consecutive days at a dose of 10 mg FF/kg bw are shown in Figure 5. The statistical analysis showed no significant difference in the B-cell counts between shrimps having received FF once-a-day or twice-a-day ($p > 0.05$). When considering the sampling time, it appeared that the hepatopancreas B-cells were significantly lower in samples collected on the third-day of medication than in those collected on day 7 after stopping medication or before medication ($p < 0.05$). Additionally, no interaction effect was observed between the doses applied and the sampling times on the numbers of B-cells ($p > 0.05$).

There was no significant difference in the number of F-cells between the applied doses (Table 5), i.e., feeding shrimp with FF-medicated feed once-a-day and twice-a-day ($p > 0.05$). Similarly, no significant different effect ($p > 0.05$) was found in the F-cell counts of shrimp across the different sampling times, i.e., there was no significant difference on day 3 of medication compared to before medication, and a similar trend was observed when comparing F-cell numbers of shrimp before medication and on day 7 after medication was stopped (Table 5). Similarly to B-cells, there was no interaction between the applied doses and the sampling times in the numbers of F-cells ($p > 0.05$). In addition, no specific histological changes, such as necrosis or vacuole formation, were observed in the hepatopancreas of shrimp during and after the medication period (Figure 4).

**Table 5.** The number of F-cells of the shrimp hepatopancreas before medication, on day 3 of medication and on day 7 after stopping medication in once-a-day and twice-a-day treatments with 10 mg FF/kg bw.

| | Number of F-Cells | | | *p*-Value |
|---|---|---|---|---|
| | **Before Medication** | **Day 3 of Medication** | **Day 7 After Medication** | |
| Once-a-day | 164 ± 7 | 150 ± 20 | 160 ± 27 | 0.439 |
| Twice-a-day | 164 ± 7 | 153 ± 17 | 169 ± 21 | |
| *p*-value | | 0.056 | | |

## 4. Discussion

### *4.1. Antimicrobial Activity of FF on Various V. parahaemolyticus Strains*

When using an agar disk diffusion method, the susceptibility of a bacterial strain to an antibiotic can be classified as "susceptible", "intermediate" and "resistant" according to the observed inhibition zone diameter, i.e., ≥18 mm, 13–17 mm and ≤12 mm, respectively [47,48]. In this study, all tested *V. parahaemolyticus* strains were susceptible (≥18 mm) to FF, except for 1 strain (belonging to Ca Mau province) with an inhibition zone diameter of 6 mm, and this strain also had an unusually high MIC (>256 µg/mL). Regarding the MIC test, the results were also varied among strains. These results illustrated that the strains of *V. parahaemolyticus* collected from different locations possess different levels of antibiotic susceptibility.

The low FF MIC values in this study were in accordance with FF MIC values against *Vibrio* spp. isolated from shrimp diseases in other studies. For example, FF MIC values were reported of between 0.5 and 4 µg/mL in Ecuador, USA, Japan and Thailand [37,57], from 0.25 to 8 µg/mL in Mexico [58], and 8 µg/mL in Ecuador by other authors [45]. In addition, the MIC values of FF in this study were low when compared to other antibiotics MIC values towards *V. parahaemolyticus* strains isolated from shrimp, with MICs for oxytetracycline, chloramphenicol, ampicillin and kanamycin of 54.5 to 88.6 µg/mL, 29.8 to 43.4 µg/mL, 89.5 to 126.6 µg/mL and 21.5 to 28.9 µg/mL, respectively [59]. Rebouças et al. [60] reported an MIC value of 400 µg/mL for oxytetracycline against *Vibrio* spp. strains. FF seems to be more effective than other antibiotics with similar bioavailability. Indeed, Rebouças et al. [60] showed using a disk diffusion method that 31 strains of *Vibrio* spp. isolated from shrimp hepatopancreas and hatchery water exhibited intermediate resistance to ampicillin, aztreonam, gentamicin, imipenem, oxytetracycline and cefoxitin, whereas FF was effective against all *Vibrio* isolates

Several previous studies reported that *V. parahaemolyticus* isolated from samples and environment sources exhibit resistance to multiple antibiotics, including amoxicillin, ampicillin, carbenicillin, colistin, gentamicin, tobramycin and the cephalosporin group, such as cefazolin, ceftazidime and cephalothin [61–64]. In the study conducted by Tran et al. [65],

pathogenic *V. parahaemolyticus* strains isolated from seafood and water samples demonstrated high levels of resistance to streptomycin (84.6% at 10 μg), sulfisoxazole (57.7% at 250 μg) and ampicillin (57.7% at 10 μg). In the study of Rocha et al. [66] on shrimp farming in Northeastern Brazil, antibiotic sensitivity tests on 70 strains of *Vibrio* isolated from water and sediment exhibited intermediate resistance to ampicillin, aztreonam, cephalothin, ceftriaxone and cefotaxime. In another study on the resistance of *V. parahaemolyticus* isolated from shrimp samples in Malaysia, *V. parahaemolyticus* isolates were resistant to some antibiotics, i.e., amikacin (26.7%), tetracycline (36.6%), ceftazidime (46.7%), cefepime (50%), cefotaxime (60%), amoxicillin-clavulanic acid (63.3%), and ampicillin (90%) [67].

Generally, the results of this study showed that *V. parahaemolyticus* strains were susceptible towards FF. That explains why this antibiotic is widely used in shrimp aquaculture to treat bacterial diseases, like necrotizing hepatopancreatitis [68]. However, inappropriate and sustained antibiotic application in food-producing environments promotes selective pressure that favors the survival and proliferation of resistant bacteria. A key mechanism in this resistance development involves the transfer of genetic material, i.e., plasmids, which carry resistance-conferring nucleotide sequences [69]. Therefore, the antimicrobial activity of FF should be evaluated regularly since this antibiotic is applied widely for shrimp disease treatment.

### *4.2. Pharmacokinetics of FF in Shrimp*

The concentration of FF in the hepatopancreas was much higher than that in plasma and muscle, and the order was hepatopancreas > plasma > muscle. This is similar to what was shown in the study of Fang et al. [36], Ren et al. [11] and Feng et al. [38], who showed shrimp hepatopancreas had the highest FF concentration. The antibiotics were absorbed directly from the shrimp stomach to the hepatopancreas after oral administration [11,70]. According to Verri et al. [71] and Faroongsarng et al. [72], the hepatopancreas plays a role in the metabolism and excretion of antibiotics in crustaceans. $T_{max}$ values of shrimp organs in the current study were shorter than those in the study of Fang et al. [36] following a single dose of 10 mg FF/kg bw in white leg shrimp by gavage feeding. The same authors reported $C_{max}$ values in the hepatopancreas, plasma and muscle of 164.22 μg/g, 5.53 μg/mL and 1.91 μg/g, respectively, and $AUC_{0-inf}$ values for corresponding organs of 871.73 mg·h/kg, 41.27 mg·h/L and 15.97 mg·h/kg, respectively. These $C_{max}$ and $AUC_{0-inf}$ values were much higher than those in the present study. Feng et al. [38] compared shrimp FF treatment by intramuscular injection and oral routes and showed that FF concentrations in plasma and tissues after oral administration were several times lower compared to those observed after FF intramuscular injection. However, the method of using antibiotics through feed (oral administration) is a commonly applied method in aquaculture in Vietnam.

After absorption and distribution, antibiotics will undergo elimination from the shrimp body. FF levels were rapidly eliminated in white leg shrimp plasma with a $T_{1/2el}$ value of 0.35 h and an apparent total body clearance of 9.75 L/kg/h. The absorption rate of antibiotics depends heavily on water temperature—the higher the water temperature, the more rapidly $T_{max}$ is reached [30,38]. For instance, in the current study, the $T_{max}$ value (1.77 h) in shrimp plasma at 26.5 °C was longer than that at 28 °C (0.5 h) [38]. There was evidence that drug absorption also depends on physiological differences between species, specifically, the structure of the intestine and the species-specific response to environmental factors [27]. For example, at the same applied dose (10 mg FF/kg bw), the $T_{max}$ value in plasma of striped catfish was 3.70 h [24], while in the present study, the $T_{max}$ value in shrimp plasma was 1.77 h. In addition, the PK parameters vary between different antibiotics, and according to the mode of drug administration and organ measurement as well as the dosage used (Table 6).

**Table 6.** PK parameters in white leg shrimp on other antibiotics according to different routes of administration.

| Antibiotic | Muscle | | | | Hepatopancreas | | | | Plasma | | | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $T_{max}$ (h) | $C_{max}$ (mg/kg) | $AUC_{0-\infty}$ (mg h/kg) | $T_{1/2el}$ (h) | $T_{max}$ (h) | $C_{max}$ (mg/kg) | $AUC_{0-\infty}$ (mg h/kg) | $T_{1/2el}$ (h) | $T_{max}$ (h) | $C_{max}$ (mg/L) | $AUC_{0-\infty}$ (mg·h/L) | $T_{1/2el}$ (h) | |
| Single dose | | | | | | | | | | | | | |
| Oxytetracyline * 100 mg/kg | 6 | 0.73 | 32.63 | 23.53 | 1 | 149.5 | 1015 | 14.89 | 6 | 27.77 | 691.3 | 11.01 | [73] |
| Oxytetracyline ** 10 mg/kg | 6 | 7.94 | 166.5 | 20.90 | - | - | - | - | 0 | 32.22 | 266.1 | 20.74 | [74] |
| Thiamphenicol * 10 mg/kg | 2 | 2.98 | 29.33 | 6.84 | 1 | 204.2 | 1381 | 31.29 | 2 | 7.96 | 43.96 | 10.66 | [36] |
| Sulfamethoxazole * 83.3 mg/kg | 1 | 21.5 | 203.9 | 6.53 | 0.5 | 89.54 | 381.1 | 8.22 | 1 | 36.52 | 395.3 | 13.76 | [75] |
| Trimethoprim * 16.7 mg/kg | 1 | 0.78 | 1.90 | 2.12 | 0.5 | 73.17 | 321.3 | 4.53 | 1 | 0.89 | 4.82 | 7.38 | [75] |
| Enrofloxacin *** 10 mg/kg | 0.25 | 5.81 | 90.1 | 52.3 | 0.5 | 11.23 | 274.2 | 75.8 | 0.083 | 4.87 | 75.8 | 19.8 | [76] |
| Multiple dose | | | | | | | | | | | | | |
| Enrofloxacin * 30 mg/kg | 1 | 1.96 | 34.75 | 10.92 | 3 | 16.29 | 314.9 | 15.86 | 1 | 19.64 | 299.6 | 16.07 | [77] |
| Oxytetracyline * 100 mg/kg | 6 | 1.67 | 66.05 | 35.39 | 6 | 124.3 | 1858 | 18.89 | 6 | 68.03 | 2049 | 20.52 | [73] |
| Sulfamethoxazole * 83.3 mg/kg | 1 | 47.8 | 403.5 | 10.92 | 1 | 73.73 | 756.5 | 11.33 | 1 | 85.89 | 1493 | 11.03 | [75] |
| Trimethoprim * 16.7 mg/kg | 1 | 0.80 | 3.03 | 2.71 | 1 | 46.50 | 190.2 | 9.65 | 1 | 1.14 | 4.84 | 5.25 | [75] |

$T_{max}$ mean time to maximal concentration (h), $C_{max}$ mean maximal concentration (mg/kg or mg/L), $AUC_{0-\infty}$ mean area under the concentration-time curve from 0 h to ∞ (mg h/kg or mg h/L), $T_{1/2el}$ mean elimination half-life of drug (h), * oral administration, ** intra-sinus administration, *** intramuscular administration.

FF is classified in the group of time-dependent antibiotic [78], which follow the time-dependent killing pattern, and according to Reed [79], these antibiotics' effectiveness is dependent on the duration of pathogen exposure to the antibiotic. The time during which the tissue concentration exceeds the MIC (T > MIC) against a bacteria should be considered as a predictor of efficacy against the corresponding target bacteria. The therapeutic dose should be calculated to maintain the concentrations higher than the MIC towards the targeted bacterial strain. According to AliAbadi and Lees [80], for time-dependent antimicrobials, the T > MIC criteria should be used, and the value must be in the range of 40% to 50% of the dosing interval to achieve efficiency of treatment. In this study, the $C_{max}$ value in shrimp plasma was 60.56 µg/L (Table 2) and much lower than the MIC values of FF towards *V. parahaemolyticus* (Table 1). In theory, this means that AHPND caused by *V. parahaemolyticus* in white leg shrimp may not be treatable with a single dose of 10 mg FF/kg bw. It means that to ensure the effectiveness of this medication, the dose should be higher than 10 mg FF/kg bw to achieve the desired FF concentration in plasma or hepatopancreas, but the toxicity of FF should then be assessed in white leg shrimp.

### *4.3. Depletion of Florfenicol in White Leg Shrimp Muscle*

Florfenicol residues were rapidly eliminated in shrimp muscle and became undetectable within 24 h after stopping medication, in both treatment frequencies (once-a-day and twice-a-day). To determine the withdrawal time, the study followed the definition of this parameter as the period required for drug residues in edible tissues to fall below a safe threshold for human consumption [44]. This calculation was based on the maximum residue limit (MRL) of 100 µg/kg for the sum concentration of FF and its metabolite (FFA), as established by the European Commission [81] and the Korean Ministry of Food and Drug Safety [82]. In this study, WT was estimated only for the twice-a-day treatment and was 27.9 degree-days (less than 2 days at 26.5 °C). Although research on FF depletion has been performed in many fish species, those studies are limited for crustaceans. When comparing with fish, the rapid depletion of FF in shrimp muscle seen in this study is similar to the observations in the study of Pham et al. [24] performed on stripped catfish with the same dosage. This is not the same for Asian seabass, as the FF residual level and WT of shrimp muscle was much lower than those reported by Rairat et al. [83] on Asian seabass, where the muscle FF concentration was 0.28 µg/g on day 4 and 0.15 µg/g on day 10 after the last FF oral dose, and the WT was estimated up to 8 days at 25 °C (approximately 204 degree-days). Besides the species, the difference may be due to the administration dose and the temperature, 10 mg/kg and 26.5 °C (in this study) compared with 15 mg/kg and 25 °C in the study of Rairat et al. [83]. The effect of temperature on depletion was also found in a study performed on tilapia, where the WT of FF at 22 °C was longer than that at 28 °C [84].

Compared to other commonly used antibiotics in white leg shrimp, FF demonstrated a relatively shorter depletion period and WT in this study. For instance, oxytetracycline administered at 4.5 g/kg feed for 14 consecutive days showed a WT of 96 h at 29 °C [44], while chloramphenicol and sulfamethoxazole, both administered at 2 g/kg feed for 3 consecutive days, showed longer WTs of 139.7 h and 30.6 h, respectively, at 24 °C [85]. These comparisons highlight the influence of the type and the dose of the applied antibiotic on residue depletion and WT in shrimp muscle. Rapid depletion of FF is consistent with the results of Huynh et al. [50] on cefotaxime administered to shrimp at 25 mg/kg bw for 3 consecutive days; the cefotaxime concentration in white leg shrimp muscle was below LOD after stopping medication for 24 h.

Generally, quick depletion of FF in shrimp muscle resulted in a short withdrawal time, suggesting that the health risk for humans consuming white leg shrimp treated with 10 mg FF/kg bw can be considered to be negligible.

### *4.4. Impact on Shrimp Hepatopancreas Histology*

The hepatopancreas has been investigated by histologists and biologists for more than a century [86]. The hepatopancreas of shrimp is constituted of many tubules. The tubule walls are composed of simple epithelium, containing four main types of cells, namely, E-cells (embryonalzellen), F-cells (fibrillenzellen), R-cells (restzellen) and B-cells (blasenzellen) [87]. In this investigation, the histology of the hepatopancreas was observed; however, only B- and F-cells were subjected to cell number counting, because the growth rate of shrimp is decreased during antibiotic application (as stated by shrimp famers), and the growth rate is related to digestive function in the shrimp hepatopancreas. According to Hu and Leung [88], B- and F-cells in the shrimp hepatopancreas play key roles in digestive functions. F-cells are responsible for synthesizing digestive enzymes, which are stored within a large vacuole. These F-cells then transform into B-cells, which subsequently release the enzymes into the hepatopancreatic lumen via a holocrine secretion process [89]. Another reason is that the proportion of R-cells in the hepatopancreas of white leg shrimp was similar in both normal and growth-retarded shrimp, while the number of B-cells was lower in growth-retarded shrimp [51]. Under a microscope, F-cells are very large with a cytoplasm that is noticeably metachromatic; B-cells are very prominent under light microscope with one or two very large vacuoles which are filled with a flocculent material and are usually so large that the nucleus is located to the periphery of the cell [86,89].

In the current study, there were no abnormal signs in the shrimp hepatopancreas histology during medication, and the cells of the shrimp hepatopancreases had no negative impacts when using FF-medicated feed at a dose of 10 mg FF/kg bw in both once-a-day and twice-a-day treatments. These results are similar to that obtained in a previous study on white leg shrimp treated with cefotaxime at 25 mg/kg bw, showing no negative impacts on shrimp hepatopancreas cells during medication [50]. Regarding other antibiotics, several studies found negative effects on shrimp hepatopancreas histology, e.g., enrofloxacin [43] or oxytetracycline [41,44], which showed that the higher the dose of antibiotics used, the more serious the impact on the hepatopancreas of shrimp.

## 5. Conclusions

This study showed quick absorption and elimination of FF in the plasma, hepatopancreas and muscle of white leg shrimp after FF once oral administration, with a short elimination half-life of 0.35 h. The FF withdrawal time in shrimp muscle was 27.9 degree-days (2 days) when FF was administered twice-a-day and using an MRL of 100 µg/kg, which will ensure the safety of shrimp products for consumers. Oral therapeutic use of FF in shrimp slightly affected the histology of shrimp hepatopancreas during medication, but the organ structure recovered after 7 days of stopping medication. A study on increased dose, however, should be considered to ensure the control of *V. parahaemolyticus* infection during shrimp culture.

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Q.V.L. and M.P.T.; project administration and funding acquisition, M.-L.S. and M.P.T. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The data presented in this study are available from the first author.

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**Conflicts of Interest:** The authors all declare that they have no conflicts of interest.

## Abbreviations

| | |
|---|---|
| AHPND | Acute hepatopancreatic necrosis disease |
| $AUC_{0-inf}$ | Area under the plasma concentration-time curve from time 0 to infinity |
| Cl | Total body clearance |
| $C_{max}$ | Maximum concentration |
| EMA | European Medicine Agency guidelines |
| FF | Florfenicol |
| IS | Internal standard |
| $k_{el}$ | Elimination rate constant |
| MIC | Minimum inhibitory concentration |
| MRL | Maximum residue limit |
| PD | Pharmacodynamics |
| PK | Pharmacokinetic |
| PK/PD | Pharmacokinetics/Pharmacodynamics |
| $T_{1/2el}$ | Elimination half-life |
| $T_{max}$ | Time to maximal plasma concentration |
| $V_d$ | Volume of distribution |
| WT | Withdrawal time |

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