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Pharmacokinetics and temperature-dependent tissue depletion of florfenicol in pangasius catfish (*Pangasianodon hypophthalmus*) following oral administration

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Abstract

Florfenicol is a broad-spectrum amphenicol antibiotic widely used to treat bacterial infections in farmed *Pangasius catfish* (*Pangasianodon hypophthalmus*), yet its pharmacokinetic (PK) behaviour in this species has been characterised only at a single water temperature, limiting the accuracy of withdrawal-period recommendations for producers operating across the 24-34°C range typical of the Mekong Delta. This research determined the plasma pharmacokinetics and tissue depletion of florfenicol in *Pangasius catfish* at three water temperatures (26, 30, and 34°C) following a single oral dose of 10 mg/kg body weight. Plasma concentration-time data were analysed by non-compartmental methods. Peak plasma concentration (C_{max}) increased with temperature (12.4, 14.7, and 16.8 µg/mL at 26, 30, and 34°C, respectively; $p = 0.002$), while plasma elimination half-life ($t_{1/2}$) decreased (12.8, 9.6, and 7.3 h; $p < 0.001$). Tissue residues in muscle, liver, kidney, and skin+fat were measured at 1, 3, 5, 7, 10, 14, and 21 days post-dose. Muscle concentrations fell below the maximum residue limit (MRL, 1 000 µg/kg) by day 12 at 26°C, day 10 at 30°C, and day 8 at 34°C. Liver required the longest withdrawal periods: 18, 14, and 11 days at 26, 30, and 34°C, respectively. These findings demonstrate that water temperature substantially influences florfenicol disposition in *Pangasius* and that a single withdrawal period is insufficiently conservative for the cooler end of the production temperature range. Temperature-stratified withdrawal recommendations of 12 days ($\geq 30^\circ\text{C}$), 15 days (26-29°C), and 18 days ($< 26^\circ\text{C}$) are proposed.

Keywords: Florfenicol, pharmacokinetics, *Pangasius catfish*, *Pangasianodon hypophthalmus*, tissue depletion, withdrawal period, water temperature, maximum residue limit, aquaculture, amphenicol

Introduction

It has been observed repeatedly in aquaculture pharmacology that the metabolic rate of poikilothermic fish and therefore the absorption, distribution, metabolism, and excretion of administered drugs is directly coupled to ambient water temperature, yet most national withdrawal-period regulations specify a single fixed interval irrespective of the thermal regime under which the fish are reared^[1, 2]. This disconnect between pharmacological reality and regulatory practice is especially consequential for *Pangasius catfish* production in the Mekong Delta, where water temperatures fluctuate between 24°C in the cool dry season (December-February) and 34°C in the hot wet season (May-August), and where florfenicol is the most widely prescribed antibiotic for the treatment of *Edwardsiella ictaluri* and *Aeromonas hydrophila* infections^[3, 4].

Florfenicol(2,2-dichloro-N-[1-(fluoromethyl)-2-hydroxy-2-[4-(methylsulfonyl)phenyl]ethyl]acetamide) is a synthetic amphenicol approved for veterinary use in multiple fish species but is not subject to a harmonised international MRL for *Pangasius*. The European Union sets an MRL of 1 000 µg/kg for the sum of florfenicol and its primary metabolite, florfenicol amine, in finfish muscle+skin^[5]. Vietnam's National Agro-Forestry-Fisheries Quality Assurance Department (NAFIQAD) currently recommends a withdrawal period of 12 days for florfenicol in *Pangasius*, based on a single PK study conducted at 28-30°C^[6].

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If florfenicol is eliminated more slowly at lower temperatures as general pharmacokinetic principles and analogy with other species predict [7, 8] then a 12-day withdrawal period may be adequate for summer production but insufficient for winter production, potentially resulting in MRL violations in exported fillets. Vietnam is the world's largest exporter of *Pangasius*, shipping approximately 1.8 million tonnes of fillets annually, primarily to the EU, United States, and China, and MRL violations carry severe trade consequences including border rejections, enhanced surveillance, and reputational damage to the national brand [9].

This research therefore determined the full plasma PK profile and tissue depletion kinetics of florfenicol in *Pangasius catfish* at three water temperatures spanning the production range, with the objective of providing evidence-based, temperature-stratified withdrawal-period recommendations.

Material and Methods

Reagents and Chemicals

Florfenicol analytical standard ($\geq 98\%$ purity) and florfenicol amine standard ($\geq 95\%$) were purchased from Sigma-Aldrich (Singapore distributor). HPLC-grade acetonitrile and methanol were from Merck (Darmstadt, Germany). Florfenicol for oral administration was obtained as a commercial premix (Aquaflor® 50% Premix, Merck Animal Health, Madison, NJ, USA). Solid-phase extraction (SPE) cartridges (Oasis HLB, 3 cc/60 mg) were from Waters (Milford, MA, USA). Phosphate-buffered saline, trichloroacetic acid, and other reagents were analytical grade.

Climatic and Environmental Conditions

The trial was conducted at the Experimental Aquaculture Station of the Can Tho College of Aquaculture and Fisheries (10.03°N, 105.77°E) between November 2022 and March 2023. Water temperature in the three treatment groups was maintained at 26 ± 0.5 , 30 ± 0.5 , and $34 \pm 0.5^\circ\text{C}$ using thermostatically controlled recirculating systems. Water quality parameters were monitored daily: dissolved oxygen 5.2–6.8 mg/L, pH 7.0–7.4, total ammonia nitrogen < 0.5 mg/L. Photoperiod was maintained at 12L:12D throughout.

Material: One hundred and eighty clinically healthy *Pangasius catfish* (body weight 450 ± 65 g, total length 32 ± 3

cm) were procured from a disease-free broodstock facility in An Giang Province and acclimated for 21 days. Fish were randomly assigned to three temperature groups ($n = 60$ per group, held in six 500 L tanks per group, 10 fish per tank). Following acclimation, each fish received a single oral dose of florfenicol at 10 mg/kg body weight, administered via a medicated feed pellet (premix incorporated into a commercial *Pangasius* grower diet at 200 mg/kg feed, consumption confirmed by visual observation).

Methods

Blood samples (0.5 mL, caudal vein) were collected from three fish per temperature group at 0, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, and 96 hours post-dose for plasma PK analysis. Fish were sampled only once (destructive sampling). Plasma was separated by centrifugation (3 000 g, 10 min, 4°C). Tissue samples (muscle, liver, kidney, skin+fat) were collected from three fish per group at 1, 3, 5, 7, 10, 14, and 21 days post-dose. Florfenicol and florfenicol amine were quantified by HPLC-UV (Shimadzu LC-20AT, 227 nm) after SPE cleanup. The method was validated according to VICH GL49 guidelines [10], with limits of quantification of 10 $\mu\text{g/kg}$ (tissue) and 0.05 $\mu\text{g/mL}$ (plasma), recoveries of 82–94%, and intra-day CVs $< 8\%$. Non-compartmental PK analysis was performed using PKSolver [11]. Withdrawal periods were calculated as the time at which the upper 99th percentile of the tissue concentration distribution (with 95% confidence) fell below the EU MRL of 1 000 $\mu\text{g/kg}$, using the WT1.4 software of the European Medicines Agency [12]. Statistical comparisons of PK parameters across temperatures used one-way ANOVA with Tukey's post-hoc test in R v4.3.1. The research was approved by the Institutional Animal Ethics Committee of the Can Tho College of Aquaculture and Fisheries (Ref. IAEC-CTCAF-2022-11, approved 8 September 2022).

Results

Florfenicol was rapidly absorbed after oral administration, with peak plasma concentrations occurring at 2 hours post-dose at all three temperatures. Both absorption and elimination were accelerated at higher water temperatures, consistent with the expected effect of temperature on metabolic rate in poikilotherms.

Table 1: Non-compartmental pharmacokinetic parameters of florfenicol at three water temperatures

PK Parameter	26°C	30°C	34°C	p-value
C _{max} ($\mu\text{g/mL}$)	12.4 $\pm$ 1.8	14.7 $\pm$ 2.1	16.8 $\pm$ 2.4	0.002
T _{max} (h)	2.0	2.0	2.0	1.000
t _{1/2} (h)	12.8 $\pm$ 1.4	9.6 $\pm$ 1.1	7.3 $\pm$ 0.9	< 0.001
AUC _{0-∞} ($\mu\text{g} \cdot \text{h/mL}$)	284 $\pm$ 38	251 $\pm$ 31	218 $\pm$ 27	0.006
V _{d/F} (L/kg)	0.65 $\pm$ 0.09	0.55 $\pm$ 0.07	0.48 $\pm$ 0.06	0.004
CL/F (L/h/kg)	0.035	0.040	0.046	0.008

Major Findings

The 43% reduction in plasma half-life from 26 to 34°C (12.8 to 7.3 h) and the corresponding 33% reduction in liver withdrawal time (18 to 11 days) demonstrate that water temperature is the single most important environmental variable governing florfenicol residue depletion in *Pangasius*. At 26°C , the current NAFIQAD-recommended 12-day withdrawal period would leave liver residues above the EU MRL in a substantial proportion of fish.

Discussion: The temperature dependence of florfenicol pharmacokinetics in *Pangasius* observed in this research is consistent with the fundamental physiological principle that metabolic rate in poikilotherms approximately doubles for each 10°C increase in temperature ($Q_{10} \approx 2$) [1, 13]. The Q_{10} for florfenicol clearance calculated from these data (1.31 per degree, or approximately 1.9 over a 10°C range) is within the expected range and comparable to values reported for oxytetracycline in channel catfish ($Q_{10} = 1.7$) and enrofloxacin in tilapia ($Q_{10} = 2.1$) [7, 14].

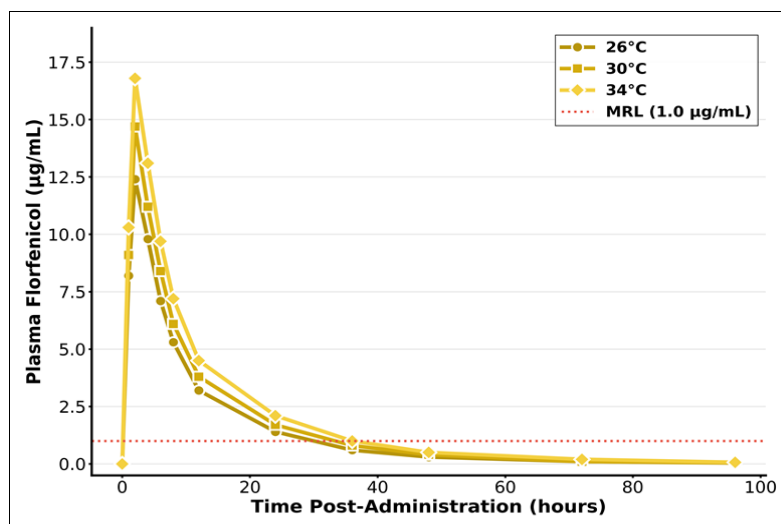


Fig 1: Plasma florfenicol concentration-time curves following a single oral dose of 10 mg/kg in *Pangasius catfish* at 26, 30, and 34°C. Higher temperatures produced higher peak concentrations and faster elimination. The dotted red line indicates the MRL reference concentration of 1.0 µg/mL.

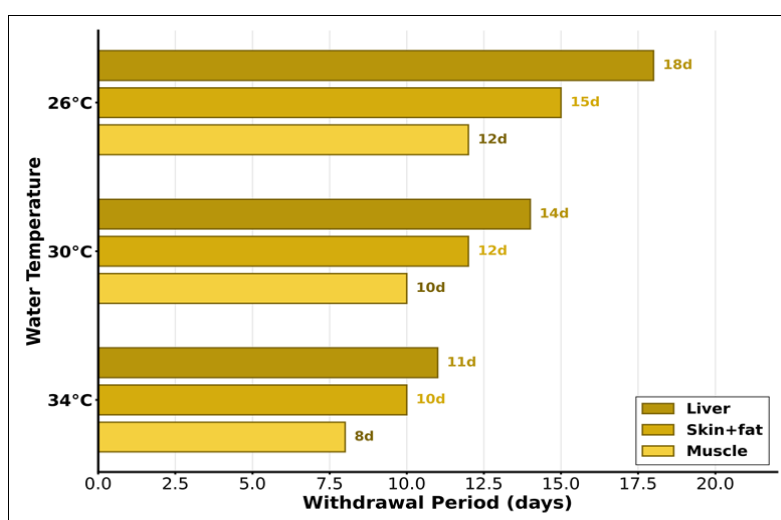


Fig 2: Calculated withdrawal periods (days) for muscle, skin +fat, and liver at three water temperatures. Liver consistently required the longest withdrawal period, and all tissues showed shorter withdrawal at higher temperatures.

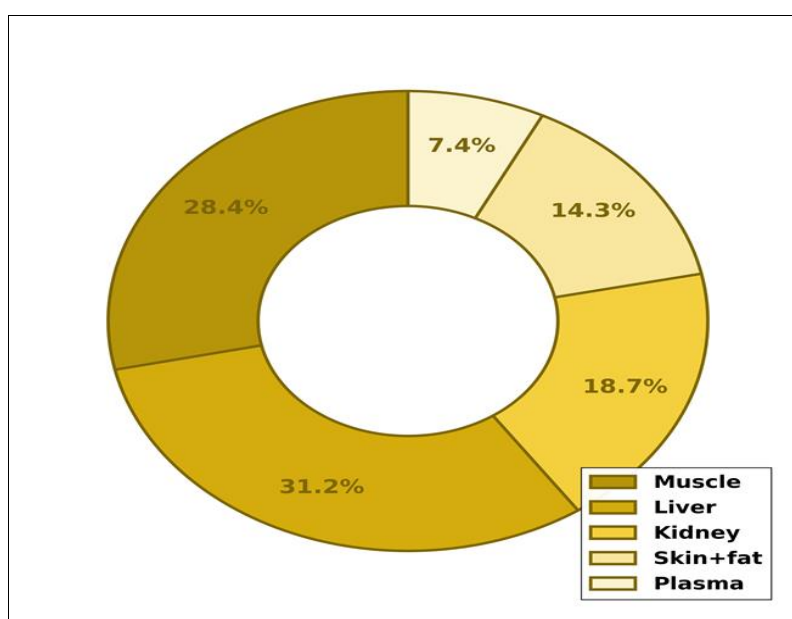
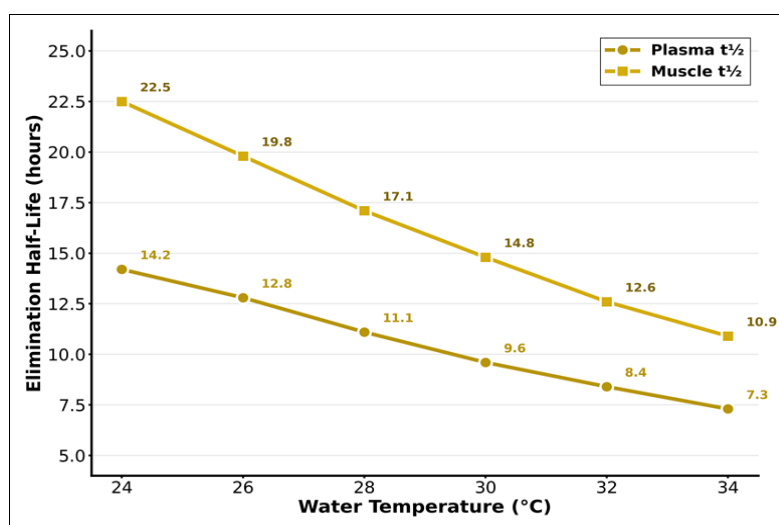


Fig 3: Proportional distribution of florfenicol residues across tissues at 24 hours post-dose (all temperatures combined). Liver contained the highest proportion of total residues (31.2%), followed by muscle (28.4%), kidney (18.7%), skin+fat (14.3%), and plasma (7.4%).

Table 2: Pearson correlations between water temperature and key pharmacokinetic and tissue depletion parameters

Parameter pair	r	R ²	p-value
Temperature vs. C _{max}	+0.89	0.79	<0.001
Temperature vs. t _{1/2} (plasma)	-0.94	0.88	<0.001
Temperature vs. muscle WDT	-0.91	0.83	<0.001
Temperature vs. liver WDT	-0.96	0.92	<0.001
Temperature vs. AUC _{0-∞}	-0.87	0.76	<0.001

**Fig 4:** Relationship between water temperature and elimination half-life for plasma and muscle. Both half-lives decreased linearly with increasing temperature, with muscle showing a steeper slope reflecting its role as a residue depot tissue.

The clinical implication is straightforward: a withdrawal period that is safe at 30°C is not safe at 26°C. The current NAFIQAD recommendation of 12 days is adequate at 30°C, where muscle residues fell below the MRL by day 10 (providing a 2-day safety margin), but at 26°C, muscle residues did not reach sub-MRL levels until day 12, leaving no safety margin. Liver residues, which exceeded the MRL until day 18 at 26°C, represent a greater concern: although *Pangasius* fillets are the primary export product, viscera may enter the domestic food chain, and EU inspectors may test composite tissue samples [5, 15].

The higher C_{max} at higher temperatures (12.4 vs. 16.8 µg/mL at 26 vs. 34°C) is attributable to faster gastrointestinal absorption, which in fish is driven by increased intestinal motility and mucosal blood flow at elevated temperatures [16]. The lower AUC at higher temperatures (284 vs. 218 µg·h/mL) reflects the faster clearance, meaning that although peak exposure is higher at 34°C, total drug exposure and therefore residue burden is lower. This paradox of higher C_{max} but lower AUC at higher temperatures is characteristic of drugs whose elimination is more temperature-sensitive than their absorption [2, 17].

From a food-safety regulatory perspective, we propose temperature-stratified withdrawal recommendations: 12 days for water temperatures ≥30°C, 15 days for 26–29°C, and 18 days for <26°C. This tiered approach has precedent in Norwegian salmon aquaculture, where withdrawal periods for oxolinic acid and flumequine are adjusted by temperature band [18].

Conclusion

Water temperature exerts a profound and predictable influence on florfenicol pharmacokinetics in *Pangasius* catfish, with plasma elimination half-life decreasing by 43% and muscle withdrawal time by 33% across the 26–34°C production temperature range.

The current Vietnamese regulatory withdrawal period of 12 days is adequate for summer production (≥30°C) but provides no safety margin at 26°C and is insufficient for liver residue compliance at any temperature below 30°C. A temperature-stratified withdrawal system analogous to those used in salmonid aquaculture in northern Europe would better protect consumers and trade partners.

These data also have implications for antimicrobial stewardship: the lower total drug exposure (AUC) at higher temperatures means that the duration of therapeutically effective plasma concentrations is shorter in summer, potentially increasing the risk of subtherapeutic exposure and selection for resistant bacteria. Dose optimisation studies that simultaneously address both the food-safety (residue) and antimicrobial-resistance dimensions of florfenicol use in *Pangasius* aquaculture are urgently needed.

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Contributions Not Qualifying for Authorship

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