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Temperature-dependent pharmacokinetics of florfenicol in Nile tilapia (*Oreochromis niloticus*) following single oral and intravenous administration

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ABSTRACT

Temperature-dependent pharmacokinetics (PK) have rarely been reported in tilapia despite there are popularly cultured worldwide, ranging from tropical to temperate climates. The present study aims to investigate the PK characteristics of florfenicol (FF) in Nile tilapia at 3 temperature levels (24, 28, and 32 °C) after single intravenous (IV) and oral (PO) dose of 15 mg/kg to evaluate the effects of temperature on PK. The serum concentrations of FF were analyzed by HPLC-UV method and PK characteristics were analyzed by 2-compartmental model. It was revealed that temperature has profound influences on certain PK parameters. The results from both IV and PO experiments were generally similar. Increasing water temperature from 24 to 32 °C led to significantly increased elimination rate constant (β) from 0.056 to 0.095 1/h and shortened elimination half-life ($t_{1/2\beta}$) from 12–13 h to 7–8 h. The absorption half-life ($t_{1/2Ka}$) were decreased from 2.28 to 1.18 h as well as the maximum serum concentration (C_{max}) from 23.14 to 16.71 μg/mL and time to reach C_{max} (T_{max}) from 1.40 to 0.75 h. The area under the serum concentration-time curves (AUC) were reduced by half while the clearances were doubled and the volume of distributions (Vd) were significantly increased. Our results also demonstrated that for FF in Nile tilapia the temperature coefficient (Q_{10}) values could be applied to predict the CL and β at a specific temperature with high accuracy (94–116%). The temperature-sensitive PK parameters such as the increase in Ka, Vd, and β significantly affected the serum concentrations and its overall exposure (AUC), thereby could potentially implicate that proper dosing regimen should take water temperature into consideration. The study highlighted the possibility of water temperature effect on therapeutic outcome of FF used in tilapia and likely other cichlids.

1. Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the most important aquaculture species worldwide. In 2016, world aquaculture production of tilapia and other cichlids was 5,899,000 t, second to carps and other cyprinids (30,545,000 t) as the most important farmed freshwater fish; and Nile tilapia was the dominant cichlid species accounting for about 70% (4,199,567 t) of the cichlids production (FAO, 2018). Because of eurythermal nature of tilapia, it has been cultured in a wide range of climatic zones, from tropical to temperate (Rakocy, 2005). As tilapia culture become more intensified, problems associated with bacterial infection become a real threat to the industry. The important pathogenic bacteria for tilapia include *Streptococcus agalactiae*, *S. iniae*, *Francisella noatunensis* subsp. *orientalis*, *Aeromonas hydrophila*, *Flavobacterium columnare*, *Edwardsiella tarda*, and *Pseudomonas fluorescens* which favorable growth also encompassing wide range of temperature zones (Tonguthai and Chinabut, 1997; Rakocy, 2005; Plumb and Hanson, 2011). Reducing fish stress and maintaining good water quality are important for preventing disease outbreak. However, in the event that bacterial epizootic did occur, antimicrobial drug is often the main hope for the farmer to stop massive economic loss. When used properly, antimicrobials are among the most effective measures available for controlling the outbreak.

Florfenicol (FF) is among a few antimicrobial drugs approved for treatment of food fish in many countries. The general recommended dose of FF is 10–15 mg/kg/day for 10 days (U.S. FDA, 2018) or 10 mg/kg/day for 3–5 days (Taiwan Council of Agriculture, 2017). Pharmacokinetic (PK) characteristics of FF in many fish species have already been published. However, to the best of the authors' knowledge, only 2 studies regarding PK characteristics of FF in tilapia have been reported to date (Feng and Jia, 2009; Wang et al., 2010) but none of them investigated the effect of water temperature on PK. As fishes are poikilothermic animals, their body temperature and metabolic rate are fundamentally dependent on water temperature. In fact, temperature has strong influence on the absorption and elimination rates of many antibacterial drugs including FF as reported in common carp (Oshima et al., 2004), channel

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catfish (Liu et al., 2009), Japanese eel (Lin et al., 2015), and crucian carp (Yang et al., 2018); the absorption and elimination half-lives are usually faster at warmer temperature as well as the rate of drug clearance. The dissimilarity in PK parameters among different temperature levels could inevitably have implications on the dosing regimen selection; this is because serum concentration of antimicrobial drug is influenced by certain PK parameters such as volume of distribution (Vd), elimination rate constant (β), absorption rate constant (Ka), and bioavailability (F), which can potentially be altered by changing temperature, therefore it is also likely that proper dosing regimen should be adjusted with water temperature. In addition to the economic importance of Nile tilapia, it is among the best candidates to study the effects of temperature as it can tolerate a wide range of temperature (11–40 °C) even though the preferred temperature for tilapia is between 28 and 32 °C (Teichert-Coddington et al., 1997). The aims of our study were to investigate the PK characteristics of FF in Nile tilapia reared at 3 different temperature levels, i.e., 24, 28, and 32 °C, encompassing climates range from tropical to subtropical, following intravascular (IV) and oral (PO) routes of administration. In addition, the temperature coefficient (Q_{10}) values were determined to quantitate the temperature effects on the elimination rate constant (β) and clearance (CL) for prediction and validation of the Q_{10} use in tilapia after FF medication.

2. Materials and methods

2.1. Chemicals

Florfenicol analytical standard was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade) and *N,N*-dimethylformamide were from Avantor Performance Materials (Center Valley, PA, USA). Propylene glycol was from AppliChem GmbH (Darmstadt, Germany). Sodium di-hydrogen phosphate anhydrous (NaH_2PO_4) and di-sodium hydrogen phosphate anhydrous (Na_2HPO_4) were from Panreac Química SLU (Barcelona, Spain).

2.2. Experimental fish

A total of 42 clinically healthy Nile tilapia, 500–700 g in weight were obtained from a commercial fish farm in Chiayi County, Taiwan and were kept in an outdoor concrete pond at College of Veterinary Medicine, National Chung Hsing University, Taiwan. Each individual fish was acclimatized in a 70 L-tank containing freshwater at the specified temperature (see below) for 6–7 days before drug administration. The water temperature was controlled at either 24, 28, or 32 °C by a 120 W-aquarium heater (Tzong Yang Aquarium, Taiwan) in room temperature (for 28 and 32 °C experiment) or in an air-conditioned room (for 24 °C). Dissolved oxygen (DO) was maintained at ≥ 5.0 mg/L and pH was in the range of 7.5–8.0. Temperature, DO, and pH were measured by a portable water quality meter (Lutron WA-2017SD, Lutron Electronics, Coopersburg, PA, USA). The animal study was approved by the Institutional Animal Care and Use Committee of National Chung Hsing University (IACUC approval No.: 106–134).

2.3. Drug administration and blood sample collection

FF solution (15 mg/mL) was prepared by dissolving FF reference standard powder with 200 μL of *N,N*-dimethylformamide and adjusting the volume with 1,2-propylene glycol to the final concentration. For intravascular (IV) administration, the FF solution was administered into the caudal vessel using a 22G-needle at a volume of 1 mL/kg to attain a dose of 15 mg/kg body weight. For oral (PO) administration, the FF solution (1 mL/kg) was administered by oral gavage to attain a dose of 15 mg/kg using 1 mL-syringe with 8.4 cm-stainless steel oral tube. Note that the dose of 15 mg/kg was selected based on the recommended dose of FF by U.S. FDA (2018). For a given route of administration, a total of 21 fish were used (n = 7 for each temperature level).

The blood samples were collected at 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 60, and 72 h post-administration for IV route and 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 60, and 72 h post-administration for PO route. At each time point (n = 7), about 0.40–0.45 mL of blood was drawn from caudal vessel into 1 mL-syringe with 22G needle without anticoagulant. The blood samples were allowed to clot at the room temperature and centrifuged at 3500 rpm (2191 × *g*; KN-70, Kubota, Japan) for 10 min; the supernatants (serum) were collected and kept at −20 °C until analysis. The drug administration and blood sampling processes were conducted without using anesthetic drug to avoid any potential drug-drug interaction or undesirable physiological change (Kleinow et al., 1992; Horsberg, 1994).

2.4. Sample preparation and HPLC analysis

The sample preparation and HPLC analysis of FF in the serum were modified from Kowalski et al. (2005). Briefly, the serum samples (200 μL) were extracted twice with 400 μL ethyl acetate and centrifuged at 3500 rpm (2191 × *g*) for 10 min. The ethyl acetate supernatants were combined into 50 mL-polypropylene tube and evaporated in the fume hood until completely dry (within a few hours). The residues were reconstituted with the mobile phase (see below) and filtered through 0.2 μm-nylon syringe filter. The HPLC system consisted of a pump (Waters 1525, Waters, Milford, MA, USA), UV–visible detector (Waters 2489, Waters, Milford, MA, USA), and C-18 column with 5 μm particle size, 150 × 4.6 mm (Apollo, Hichrom, UK). The mobile phase was a mixture of acetonitrile and phosphate buffer (10 mM NaH_2PO_4-Na_2HPO_4, pH 5) at 30:70 *v*/v. The flow rate was 1 mL/min; the detection wave length was 224 nm; and the injection volume was 50 μL.

To establish the calibration curves for quantification of FF concentration in the serum, the FF reference standard was spiked into blank tilapia serum at the final concentrations of 50, 100, 500 ng/mL, 1, 5, 10, and 50 μg/mL, then extracted and analyzed by the HPLC method described above. The weighting factor of $1/x^2$ was applied to improve the accuracy at the lower concentration of the calibration curve (Almeida et al., 2002; Gu et al., 2014). The matrix calibration curves was linear over the range of 50 ng/mL to 50 μg/mL with the weighted r^2 of 0.9792, the limit of detection (LOD) was 11 ng/mL and the limit of quantification (LOQ) was 33 ng/mL. The LOD and LOQ were calculated by 3.3*σ/S and 10*σ/S, respectively (σ = standard deviation of the y-intercept of the regression line; S = slope of the calibration curve). The % extraction recovery was > 85% in all cases and the precision was < 4% (for 0.5–50 μg/mL) or < 16% (for 50–100 ng/mL). The % accuracy, calculated by comparing the FF concentrations spiked into the serum (at final concentrations of 0.1, 1, and 10 μg/mL) after extraction with the corresponding reference standard concentrations, was 95.3–97.7%.

2.5. Pharmacokinetic analysis

Pharmacokinetic parameters of FF were analyzed using PKSolver 2.0 software (China Pharmaceutical University, Nanjing, China) (Zhang et al., 2010). The serum concentration-time profiles for oral administration was first fit with 1- and 2-compartmental model and for IV administration with 1-, 2-, and 3-compartmental model. The model selection was then based on visual inspection, lower values of Akaike's information criterion (AIC), and lesser coefficient of variation (%CV) of the estimated PK parameters (Yamaoka et al., 1978; Riviere, 1997; Gabrielsson and Weiner, 1999). Consequently, the 2-compartmental model was chosen for both oral and IV routes. To improve the curve-fitting during the terminal phase, a weighting scheme of 1/C was selected so that the bias can be minimized (Riviere, 1997).

The PK parameters investigated in this study were absorption rate constant (Ka), absorption half-life ($t_{1/2Ka}$), distribution rate constant (α), distribution half-life ($t_{1/2\alpha}$), elimination rate constant (β), elimination half-life ($t_{1/2\beta}$), transfer rate constant from the central (1) to

peripheral (2) compartment (k_{12}), transfer rate constant from the peripheral (2) to central (1) compartment (k_{21}), elimination rate constant from the central compartment (k_{10}), maximum serum concentration (C_{max}), time to reach C_{max} (T_{max}), area under the serum concentration-time curve (AUC), volume of distribution (Vd) during the elimination phase (Vz), Vd of the central compartment (Vc), Vd at steady-state (Vss), clearance (CL), mean residence time (MRT), and bioavailability (F).

2.6. Q_{10} calculation and application

Q_{10} (temperature coefficient) is a measure of biochemical rate change as a consequence of increasing the temperature by 10 °C. The Q_{10} was calculated as $Q_{10} = (R_2/R_1)^{10/(T2-T1)}$ where T_2 and T_1 are the temperature levels (°C) and R_2 and R_1 are the biochemical rates. In the present study, the Q_{10} values of the clearances (CL) and elimination rate constants (β) were calculated. The Q_{10} obtained from each pair of temperature levels (i.e., 24–28 °C, 28–32 °C, and 24–32 °C) were used to predict the CL and β at the third temperature. Finally, to assess the accuracy of the CL and β predicted by Q_{10} principle, the predicted parameters were compared to the corresponding original values determined from the PK analysis using the formula: % accuracy = (the Q_{10} predicted value at a specific temperature/the corresponding original value) x 100.

2.7. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 22 software (IBM Corporation, Armonk, NY, USA). The differences of PK parameters between different water temperatures were analyzed by one-way ANOVA, followed by either Bonferroni (when equal variances assumed) or Tamhane's T2 post hoc analysis (when equal variances not assumed). In the case that the assumption for parametric analysis were not met (non-normal distribution), a nonparametric Kruskal-Wallis test was used. Specifically, for IV study, the α, $t_{1/2\alpha}$, Vss, k_{12}, k_{10}; and for PO study the Ka, α, AUC, and k_{12} were analyzed by the Kruskal-Wallis method whereas all the other parameters in respective study were analyzed by one-way ANOVA.

3. Results

3.1. Effects of water temperature on the PK following the IV route

The serum concentrations of FF versus time after IV administration were shown in Fig. 1 and the PK parameters were presented in Table 1. Increasing water temperature from 24 to 32 °C led to significantly increased β from 0.056 to 0.095 1/h and decreased the corresponding $t_{1/2\beta}$ from 12.44 to 7.32 h. The AUC were reduced by half from 363.23 to 174.81 h·μg/mL, while the CL were doubled from 0.042 to 0.087 L/kg/h. The MRT were shortened from 15.73 to 9.06 h. The Vz at 32 °C (0.93 L/kg) were also significantly greater than that at 24 °C (0.75 L/kg).

3.2. Effects of water temperature on the PK following the PO route

The serum concentrations of FF versus time after PO administration and their PK parameters were shown in Fig. 2 and Table 2, respectively. In general, the trend and magnitude of water temperature effects were similar to those of the IV route; the β, CL, and Vz were significantly increased at the warmer temperature whereas the $t_{1/2\beta}$, AUC, and MRT were decreased. In addition to the aforementioned parameters, the decrease in $t_{1/2Ka}$, C_{max}, and T_{max} and the increase in Vc and Vss at 32 °C were also evident. At each temperature level, when the PK parameters between IV and PO studies were compared by the independent *t*-test, the CL, Vz, Vss, β, and $t_{1/2\beta}$ showed no significant difference ($P > .05$) (data not shown). The oral bioavailability (F) were in the range of 70–90% and appeared independent of water temperature.

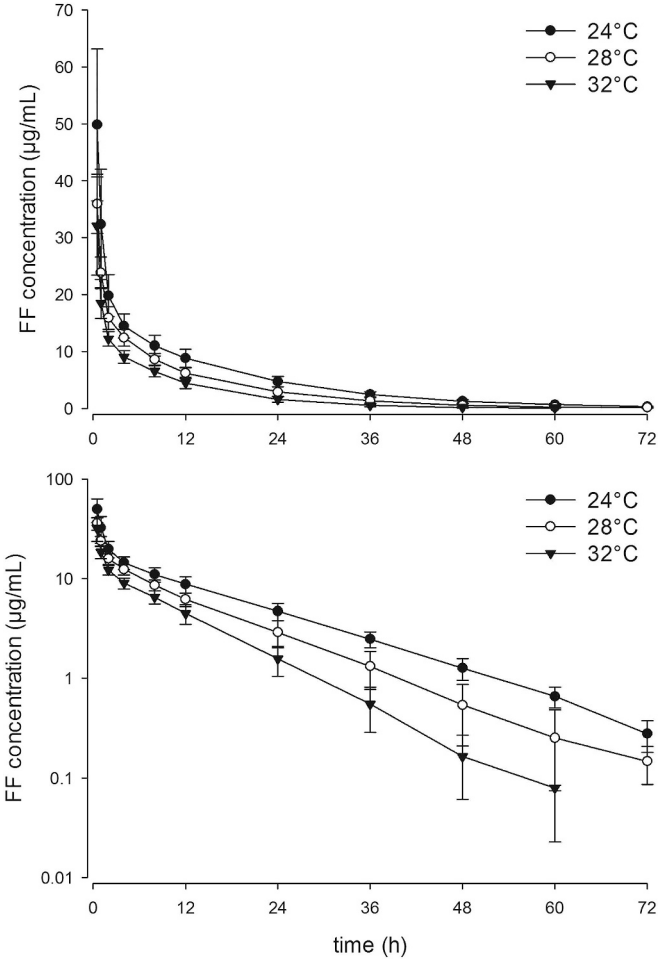

Fig. 1. Linear (above) and semi-logarithmic plots (below) of serum concentration-time profile (mean ± SD) of 15 mg/kg FF following IV administration at 3 temperatures (n = 7). Note that the FF concentrations of 5 out of 7 fish at 32 °C declined below the LOQ at 60 h post-administration.

Table 1
Pharmacokinetic parameters (mean ± SD) of 15 mg/kg FF following IV administration at 3 temperatures (n = 7).

PK parameters	24 °C	28 °C	32 °C
α (1/h)	1.55 ± 0.38[a]	1.87 ± 0.67[a]	2.57 ± 1.53[a]
$t_{1/2\alpha}$ (h)	0.45 ± 0.13[a]	0.37 ± 0.26[a]	0.27 ± 0.13[a]
β (1/h)	0.056 ± 0.004[a]	0.073 ± 0.011[b]	0.095 ± 0.015[c]
$t_{1/2\beta}$ (h)	12.44 ± 0.91[a]	9.45 ± 1.46[b]	7.32 ± 1.11[c]
k_{12} (1/h)	0.98 ± 0.30[a]	1.16 ± 0.52[a]	1.63 ± 1.22[a]
k_{21} (1/h)	0.38 ± 0.13[a]	0.43 ± 0.15[a]	0.49 ± 0.14[a]
k_{10} (1/h)	0.25 ± 0.10[a]	0.35 ± 0.23[a]	0.54 ± 0.36[a]
AUC (h·μg/mL)	363.23 ± 46.90[a]	260.36 ± 43.82[b]	174.81 ± 23.43[c]
Vz (L/kg)	0.75 ± 0.08[a]	0.81 ± 0.08[ab]	0.93 ± 0.09[b]
Vc (L/kg)	0.19 ± 0.07[a]	0.21 ± 0.10[a]	0.22 ± 0.12[a]
Vss (L/kg)	0.66 ± 0.09[a]	0.69 ± 0.12[a]	0.78 ± 0.12[a]
CL (L/kg/h)	0.042 ± 0.005[a]	0.059 ± 0.011[b]	0.087 ± 0.011[c]
MRT (h)	15.73 ± 1.91[a]	11.86 ± 2.62[b]	9.06 ± 1.76[b]

The means of half-lives are harmonic mean whereas the means of the other PK parameters are arithmetic mean. Means with different superscripts in each row are significantly different from each other ($P < .05$).

3.3. Q_{10} calculation and application

The Q_{10} concept was applied to quantitate the temperature effects on the 2 important PK parameters that have units of "rate", namely, CL (L/kg/h) and β (1/h). The results revealed that the CL exhibited higher

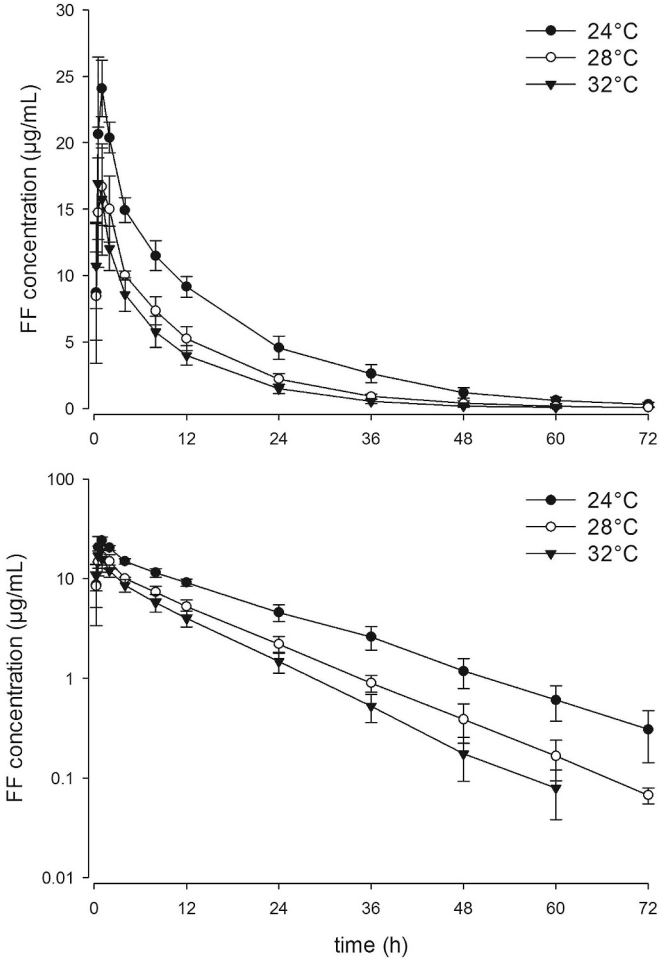

Fig. 2. Linear (above) and semi-logarithmic plots (below) of serum concentration-time profile (mean ± SD) of 15 mg/kg FF following PO administration at 3 temperatures (n = 7). Note that the FF concentrations of all fish at 32 °C declined below the LOQ at 72 h post-administration.

Table 2
Pharmacokinetic parameters (mean ± SD) of 15 mg/kg FF following PO administration at 3 temperatures (n = 7).

PK parameters	24 °C	28 °C	32 °C
Ka (1/h)	1.18 ± 0.48^{a}	1.89 ± 0.94^{ab}	2.28 ± 0.60^{b}
$t_{1/2Ka}$ (h)	0.59 ± 0.22^{a}	0.37 ± 0.20^{ab}	0.30 ± 0.09^{b}
α (1/h)	1.09 ± 0.43^{ab}	0.74 ± 0.20^{a}	1.49 ± 0.48^{b}
$t_{1/2\alpha}$ (h)	0.63 ± 0.23^{ab}	0.94 ± 0.36^{a}	0.47 ± 0.18^{b}
β (1/h)	0.055 ± 0.006^{a}	0.074 ± 0.007^{b}	0.088 ± 0.010^{c}
$t_{1/2\beta}$ (h)	12.49 ± 1.44^{a}	9.40 ± 0.89^{b}	7.90 ± 0.93^{b}
k_{12} (1/h)	0.59 ± 0.28^{a}	0.35 ± 0.20^{a}	0.59 ± 0.43^{a}
k_{21} (1/h)	0.41 ± 0.14^{a}	0.38 ± 0.06^{a}	0.65 ± 0.38^{a}
k_{10} (1/h)	0.15 ± 0.02^{a}	0.15 ± 0.04^{a}	0.20 ± 0.07^{a}
C_{max} (µg/mL)	23.14 ± 2.99^{a}	16.74 ± 2.62^{b}	16.71 ± 3.38^{b}
T_{max} (h)	1.40 ± 0.45^{a}	1.18 ± 0.32^{ab}	0.75 ± 0.36^{b}
AUC (h·µg/mL)	327.76 ± 44.07^{a}	181.54 ± 20.12^{b}	140.89 ± 22.67^{b}
Vz (L/kg)	0.75 ± 0.06^{a}	0.79 ± 0.07^{a}	1.00 ± 0.16^{b}
Vc (L/kg)	0.29 ± 0.03^{a}	0.40 ± 0.09^{b}	0.50 ± 0.24^{ab}
Vss (L/kg)	0.68 ± 0.04^{a}	0.71 ± 0.06^{a}	0.91 ± 0.14^{b}
CL (L/kg/h)	0.042 ± 0.005^{a}	0.058 ± 0.007^{b}	0.087 ± 0.012^{c}
MRT (h)	17.52 ± 2.08^{a}	13.00 ± 1.33^{b}	10.87 ± 0.97^{b}
F (%)	90.24	69.73	80.60

The means of half-lives are harmonic mean whereas the means of the other PK parameters are arithmetic mean. Means with different superscripts in each row are significantly different from each other ($P < .05$).

thermal sensitivity (i.e., had greater Q_{10} values which was in the range of 2.32–2.69) than the β (Q_{10} = 1.54–2.03), as shown in Table 3. The Q_{10} values obtained from different route of administrations were similar. The Q_{10} determined from the CL at temperatures of 28 and 32 °C (after IV) and its application to predict the CL at 24 °C were given in Table 3 as an example.

4. Discussion

It has long been known that fish's body temperature is generally the same as the surrounding water (Clausen, 1934; Morrow and Mauro, 1950) and temperature has influences on fish's physiology in several aspects such as enhancing metabolic rate (Clarke and Johnston, 1999; Killen et al., 2010), increasing cardiac output and reducing blood circulation time (Barron et al., 1987). The present study revealed profound effects of water temperature on several PK parameters of FF in Nile tilapia. For instance, increasing water temperature from 24 to 32 °C resulted in significant shortening of T_{max} and $t_{1/2Ka}$ after PO administration which suggested faster drug absorption. Similar results were also reported in many studies with different drugs and fishes, for example, FF in channel catfish (Liu et al., 2009), common carp (Oshima et al., 2004) and Japanese eel (Lin et al., 2015). To the best of the authors' knowledge, the temperature effect on the pharmacokinetics of FF in Nile tilapia has not been investigated so far except for one study containing partial information relating to the effect of temperature on muscle/skin residue of FF in Nile tilapia (Kosoff et al., 2009). The result was in general consistent with our finding in that increasing temperature fastens the drug depletion shown by the shortened tissue half-life.

Regarding C_{max} and AUC, our results clearly demonstrated that both PK parameters were decreased at higher temperature. However, unlike T_{max} and $t_{1/2Ka}$, the effects of temperature on C_{max} and AUC appeared more variable with different fishes. Some studies reported the greater values of C_{max} and/or AUC with warming water temperature such as FF in common carp (Oshima et al., 2004) and Japanese eel (Lin et al., 2015), others showed opposite trends with decreased AUC such as FF in channel catfish (Liu et al., 2009) and crucian carp (Yang et al., 2018). These seemingly contradictory results can be explained by different performance in related PK parameters. Although most of the studies demonstrated faster drug absorption at higher temperature such that the C_{max} and AUC is increased consequently, the increasing drug elimination at warmer water could at least partially negate this effect. In fact, one of the most consistent findings in fish PK studies at different temperatures, regardless of drugs and fish species, was the shortening of the $t_{1/2\beta}$ as a consequence of raising water temperature (Nouws et al., 1993; Oshima et al., 2004; Ding et al., 2006; Liu et al., 2009; Lin et al., 2015; Yang et al., 2018). Nevertheless, it should be emphasized that warming water temperature may not always result in decreasing $t_{1/2\beta}$, in the case that Vd was greater increased compared to CL, the $t_{1/2\beta}$ can be longer (Hayton, 1999). Temperature can affects CL and Vd in several ways. Increasing water temperature would result in enhanced blood flow (and thus blood flow-dependent clearance) as well as drug metabolizing enzyme activity, leading to enhanced CL of the drug (Hayton, 1999) as shown in our results and previous studies (Liu et al., 2009; Yang et al., 2018). In contrast to the CL, the Vd may or may not change with temperature. When the drug distribution involves temperature-sensitive equilibria such as reversible binding to proteins and distribution between lipid and aqueous phase, the Vd may change with temperature (Hayton, 1999). Not surprisingly, the effects of temperature on Vd of aquaculture drugs reported in the literature were diverse; some (including the current study) reported the increased Vd at warmer temperature (Nouws et al., 1993; Ding et al., 2006), others found decreased (Lin et al., 2015; Yang et al., 2018) or unchanged (Liu et al., 2009) in this parameter. These observations highlighted the importance of conducting separate temperature effect studies on target drug and fish species as temperature can have different degrees of impact on PK

Table 3
Determination of Q_{10} values for the clearance (CL) and elimination rate constant (β) and their application to predict CL and β at a specific temperatures.

PK parameters		Prediction at 24 °C			Prediction at 28 °C			Prediction at 32 °C		
			IV	PO		IV	PO		IV	PO
CL	Determination of Q_{10} for CL[a]	$CL_{32°C}$	0.087	0.087	$CL_{32°C}$	0.087	0.087	$CL_{28°C}$	0.059	0.058
		$CL_{28°C}$	0.059	0.058	$CL_{24°C}$	0.042	0.042	$CL_{24°C}$	0.042	0.042
		$T_{32°C}$	32	32	$T_{32°C}$	32	32	$T_{28°C}$	28	28
		$T_{28°C}$	28	28	$T_{24°C}$	24	24	$T_{24°C}$	24	24
		$Q_{10\ (28→\ 32)}$	2.63	2.69	$Q_{10\ (24→\ 32)}$	2.50	2.50	$Q_{10\ (24→\ 28)}$	2.38	2.32
	Prediction of CL at a specific temperature[b]	Predicted $CL_{24°C}$	0.040	0.039	Predicted $CL_{28°C}$	0.060	0.060	Predicted $CL_{32°C}$	0.084	0.082
	Original values of CL[c]	Original $CL_{24°C}$	0.042	0.042	Original $CL_{28°C}$	0.059	0.058	Original $CL_{32°C}$	0.087	0.087
	Accuracy of prediction[d]		96%	94%		102%	103%		96%	94%
β	Determination of Q_{10} for β[a]	$β_{32°C}$	0.095	0.088	$β_{32°C}$	0.095	0.088	$β_{28°C}$	0.073	0.074
		$β_{28°C}$	0.073	0.074	$β_{24°C}$	0.056	0.055	$β_{24°C}$	0.056	0.055
		$T_{32°C}$	32	32	$T_{32°C}$	32	32	$T_{28°C}$	28	28
		$T_{28°C}$	28	28	$T_{24°C}$	24	24	$T_{24°C}$	24	24
		$Q_{10\ (28→\ 32)}$	1.89	1.54	$Q_{10\ (24→\ 32)}$	1.94	1.77	$Q_{10\ (24→\ 28)}$	1.99	2.03
	Prediction of β at a specific temperature[b]	Predicted $β_{24°C}$	0.057	0.064	Predicted $β_{28°C}$	0.073	0.070	Predicted $β_{32°C}$	0.097	0.101
	Original values of β[c]	Original $β_{24°C}$	0.056	0.055	Original $β_{28°C}$	0.073	0.074	Original $β_{32°C}$	0.095	0.088
	Accuracy of prediction[d]		102%	116%		99%	95%		102%	116%

[a] The Q_{10} values were calculated from the formula: $Q_{10} = (R_2/R_1)^{10/(T2-T1)}$ where R_1 and R_2 are the rate at temperature 1 (T_1) and 2 (T_2), respectively.
[b] The prediction of CL and β were calculated from the formula: $R_1 = R_2/[Q_{10}^{(T2-T1)/10}]$ where R_1 is the predicted value at temperature 1 (T_1) and R_2 is the original values at temperature 2 (T_2). For example, the prediction of CL at 24 °C (after IV) was = $CL_{28°C}/[Q_{10}^{(T28-T24)/10}] = 0.059/[2.63^{(28-24)/10}] = 0.040$ L/kg/h.
[c] The original values of CL and β were those determined from the PK studies (Tables 1 & 2).
[d] The accuracy of the predicted CL and β were calculated from the formula: % accuracy = (the predicted value at a specific temperature/the corresponding original value)*100. For example, the accuracy of predicted CL at 24 °C (after IV) was = (0.040/0.042)*100 = 96%.

parameters for specific drug and fish, such that overall drug exposure (AUC) cannot be universally predicted.

The change in certain PK parameters due to temperature effects can be largely attributed to the increasing metabolic rate at higher temperature. In general, when temperature increases by 10 °C, the metabolic rate of the fish will approximately double (i.e., $Q_{10} \approx 2$) (Clarke and Johnston, 1999; Killen et al., 2010), which is also true for tilapia (Ross, 2000). In the present study, the Q_{10} concept was applied to quantitate the extent of temperature effects on CL and β, and to predict the PK parameters at a specified temperature. Our results revealed the practical application of Q_{10} principle. The Q_{10} values calculated from the rates at any pair of temperature levels can be used to predict the CL and β at the third temperature with high accuracy (94–116%). For example, without any further experiment, we can estimate the rate of drug elimination (judged by the CL and β) at 26 or 30 °C in which those data were unavailable, provided that the Q_{10} value and the CL and β of at least one temperature were known. The example of Q_{10} calculation and its application to predict the CL were provided in Table 3. The extent of drug distribution at a specific temperature can also be estimated by the relationship of Vz = CL/β.

The changes in CL and β as a function of temperature for a given species can be explained partially by thermodynamic principle. In short, the increase in the rate of reaction is mainly due to an increase in the kinetic energy (E_k) of the colliding molecules so that fraction of molecules that exceed the activation energy (E_a) is also increased (Schulte, 2011). Should this principle be universally true, we would expect to see increase in CL and β as temperature raised regardless of fish species and their habitats. However, based on the data from the current study and available published papers that examined PK characteristics of FF in a variety of fishes following IV route, namely, channel catfish (Gaunt et al., 2012), amur catfish (Park et al., 2006), Atlantic salmon (Martinsen et al., 1993; Horsberg et al., 1996), turbot (Ocenda et al., 2017), olive flounder (Lim et al., 2010, 2011), and cod (Samuelsen et al., 2003) at preferred temperature for a given species, there were no significant correlation between temperature and CL (Pearson correlation = 0.364, P = .301) or β (Pearson correlation = 0.619, P = .056) across different fish species (data not shown). Most of the CL and β of FF reported were around 0.03–0.07 L/kg/h and 0.03–0.06 1/h, respectively, despite difference in water temperature. In other words, the CL and β of cold water fish and warm water fish reared at their preferred temperature tended to have rather similar values than expectation from thermodynamic point of view. Note that the only IV data at 28 and 32 °C from the present study were included in the correlation analysis as these 2 temperatures are considered the optimal range for tilapia (Teichert-Coddington et al., 1997). The lack of correlation suggested that thermodynamic theory could not be applied beyond a single species; increasing temperature does not always result in increase of the CL and β when compared across species. Instead, this phenomenon can be explained by evolutionary trade-off theory in which the overall thermal sensitivity is reduced by evolutionary adaptation. Specifically, different fish species seems to have evolutionary adaptation to a particular temperature range so that the activity of metabolizing enzymes are relatively independent of temperature. In other words, the optimal temperature for enzyme activity is the temperature that fish normally found (Clarke and Fraser, 2004). The fact that between-species Q_{10} (1.83) was lower than within-species Q_{10} (2.40) for resting metabolic rate also supports this theory (Clarke and Johnston, 1999). Physiological mechanisms for adaptation to low temperature in order to compensate for reduced metabolic activity include change in unsaturation of membrane phospholipid or increase in mitochondrial volume density (e.g., in moderately active demersal fish that living in permanently cold water) (Johnston and Dunn, 1987; Guderley, 2004).

Because water temperature is believed to exert influence on PK mainly through changing the fish's metabolic rate and the metabolic rate is dissimilar among different taxonomic groups (in descending order, Gadiformes > Salmoniformes > Perciformes > Cypriniformes > Anguilliformes) (Clarke and Johnston, 1999), life-styles (pelagic > benthopelagic > benthic > bathyal), and swimming modes (thunniform > carangiform > subcarangiform > angulliform) (Killen et al., 2010), so the extent of temperature effect on PK parameters is not expected to be the same for fishes as diverse as cod and eel; thus, a general conclusion applicable to every fish species could not be drawn. On the contrary, it is possible that effect of water temperature on PK of FF reported in the current study might hold true for a fish similar phylogenetically and ecologically to Nile tilapia, but this speculation need to be verified in the future. Nevertheless, regardless of the extent of temperature effect on different fish species, it was undisputable that

temperature have significant influence on PK parameters of FF (including those implicated in dosing regimen selection such as Ka, Vd, and β) not only in tilapia but also in several fishes as shown in previous studies (Oshima et al., 2004; Liu et al., 2009; Lin et al., 2015; Yang et al., 2018).

5. Conclusions

In conclusion, our results demonstrated the profound effects of water temperature on pharmacokinetic characteristics of florfenicol in Nile tilapia. The drug was absorbed and eliminated significantly faster at 32 °C compared to 28 and 24 °C. The C_{max}, T_{max}, AUC, and MRT were significantly reduced at the higher temperature whereas the reverse was true for the Vd and CL. Since several important PK parameters were proven temperature-sensitive in this study, it is feasible to assume that temperature-dependent dosing regimen might be necessary and warrant further study. Provided that the $t_{1/2\beta}$, C_{max}, and AUC were significantly reduced at higher temperature, it was feasible to deduce that a higher dose of FF may be required at warmer water to achieve therapeutic effectiveness, a reasonable dosing adjustment scheme warrants further study.

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