Aquaculture 512 (2019) 734279

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Pharmacokinetics of florfenicol in lumpfish (*Cyclopterus lumpus* L.) after a single oral administration

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ARTICLE INFO

Keywords:
Lumpfish
Lumpsucker
Florfenicol
Pharmacokinetics
MIC
Cyclopterus lumpus

ABSTRACT

Farming of lumpfish for biological removal of sea lice from farmed Atlantic salmon has expanded rapidly in Europe and Canada over the last 5–6 years and the lumpfish has become an economically important species. There are, however, health challenges associated with bacterial diseases. In recent years, there has been an increase in antibacterial treatments prescribed for this fish species despite a lack of knowledge regarding pharmacokinetics and effect of treatment with different antibiotics.

The present study examined the uptake, tissue distribution, metabolism and elimination of the antibacterial agent florfenicol in lumpfish (*Cyclopterus lumpus* L.) following a single oral administration of 10 mg/kg fish given in feed. Plasma, head kidney, liver and muscle from six fish were sampled at each time point and analysed by liquid chromatography/mass spectrometry (LC-MS). Absorption was moderate for this drug characterised by a calculated peak plasma concentration (C_{max}) of 3.55 μg/ml obtained after 21.2 hours (T_{max}) and the elimination halflife ($t_{1/2}\beta$) relatively extended in plasma at 30 hours. Area under curve (AUC) and AUC from 0 to 24 hours ($AUC_{0\text{-}24h}$) were calculated to be 248 and 61 h μg/ml, respectively. C_{max} was calculated to 2.99 μg/g in muscle, 2.54 μg/g in liver and 4.70 μg/g in head kidney with corresponding T_{max} of 22.1, 26.4 and 19.4 h, respectively. The main metabolite, florfenicol-amine was found in low concentrations in plasma and all tissues examined. The minimum inhibition concentrations (MIC) for florfenicol of 28 of *Aeromonas salmonicida* isolates from diseased lumpfish ranged from 0.39 to 1.56 μg/ml.

The pharmacokinetical data presented here make an important basis for efficient antibacterial treatment for lumpfish using florfenicol and for calculation of suitable withdrawal time. Knowledge of florfenicol pharmacokinetics, combined with determination of antibiotic resistance among fish pathogenic bacteria and the effect of antibacterial agents on diseased lumpfish *in vivo* are important for the welfare of lumpfish and prevention of resistant bacteria.

1. Introduction

Consumption of antibacterial agents has been low in Norwegian aquaculture for the last 25 years, (www.fhi.no), largely due to the widespread use of effective vaccines in salmonid farming. Antibacterial treatment remains, however, an appropriate tool for control of infection in marine fish species for which adequate vaccines have not yet been developed, and there has been an increase in the number of prescriptions registered in recent years for treatment of lumpfish (Grave and Helgesen, 2018).

The use of cleaner-fish has increased significantly during the last decade due to increased resistance to therapeutics utilised for salmon lice (*Lepeophtheirus salmonis* Krøyer, 1837) removal and a desire for more environmentally friendly production of Atlantic salmon (*Salmo salar* L.). Traditionally, corkwing wrasse (*Symphodus melops* L.), ballan wrasse (*Labrus bergylta* Ascanius, 1767) and goldsinny wrasse (*Ctenolabrus rupestris* L.) have been used for this purpose. Lumpfish (*Cyclopterus lumpus* L.) have, however, been found to efficiently remove salmon lice at a wider range of temperatures than the wrasse species (Imsland et al., 2014; Powell et al., 2018). All lumpfish utilised as cleaner fish are cultured and production has increased from around 0.4 million fish in 2012 to over 30 million in 2017 (http://www.fiskeridir.no).

Like other farmed fish, lumpfish are susceptible to various bacterial

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https://doi.org/10.1016/j.aquaculture.2019.734279
Received 21 November 2018; Received in revised form 1 July 2019; Accepted 3 July 2019
Available online 04 July 2019

infections e.g. *Pseudomonas anguilliseptica, Vibrio ordalii, Vibrio anguillarum, Aeromonas salmonicida* and *Pasteurella* sp. (Alarcón et al., 2016; Gulla and Bornø, 2018; Ellul et al., 2018). Outbreaks of disease often occur in small fry and after stressful events such as vaccination and sea-transfer. Mortality levels up to 100% have been reported (Gulla and Bornø, 2018). Efforts to further develop efficient vaccines for lumpfish is ongoing. Currently available vaccines, which include *V. anguillarum* and atypical *A. salmonicida*, give high protection against vibriosis and trials have shown promising results after challenge with atypical *A. salmonicida* (Rønneseth et al., 2017; Haugland et al., 2018). Lumpfish cannot be intraperitoneally vaccinated before they reach 8-10 g and thus are vulnerable to infections during the early stages of culture.

Today, the two antibacterial agents commercially available as medicated feeds in Norway are oxolinic acid and florfenicol. Florfenicol is a synthetic drug with potent activity against several fish pathogenic bacteria (Fukui et al., 1987; Inglis and Richards, 1991) and is reported to have good effect on bacterial infections in Atlantic salmon (Samuelsen et al., 1998), Atlantic cod (*Gadus morhua* L.) (Samuelsen and Bergh, 2004; Seljestokken et al., 2006), channel catfish (*Ictalurus punctatus* Rafinesque, 1818) (see Gaunt et al., 2010) and Nile tilapia (*Oreochromis niloticus* L.) (see Soto et al., 2013). Furthermore, florfenicol possess excellent pharmacokinetic properties in species like Atlantic salmon (Martinsen et al., 1993; Horsberg et al., 1996) and Atlantic cod (Samuelsen et al., 2003) although in turbot (*Scophthalmus maximus* L.) the drug has a less favourable pharmacokinetic profile (de Ocenda et al., 2017).

The design of treatment regimens and prediction of probable clinical outcomes represents a practical application of pharmacokinetic data. To establish a correct dosage regime and thereby promote optimal use of an antibacterial agent, knowledge of the susceptibility of pathogen to the compound in question and the pharmacokinetic properties of the drug are required. However, as the properties of antibacterial agents can vary significantly between fish species, the pharmacokinetics of a drug should be investigated in the species in which it is intended to be used. For food-fish species, information relating to elimination time is important for the determination of a suitable withdrawal time.

The aim of this study was to examine the pharmacokinetic properties of florfenicol in lumpfish following a single oral administration, and to relate the data to the minimum inhibitory concentration (MIC) values of florfenicol to *A. salmonicida* strains, isolated from diseased lumpfish.

2. Materials and methods

2.1. Experimental fish

Unvaccinated lumpfish (*Cyclopterus lumpus* L.) were obtained from Fjord Forsk AS (Sogndal, Norway), transported to the Aquatic and Industrial Laboratory (ILAB), Bergen, Norway, and kept in flow through storage tanks (500 l) until the fish reached a mean weight of 113.5 ± 25.0 g and a length of 11.7 ± 1 cm. The seawater had a salinity of 34‰, a temperature of 12.0 ± 0.5 °C and a flow-rate of approximately 1000 l/h. The fish were fed a non-medicated ration of 1% body weight per day of dry pellets (Amber Neptun, 1.5 mm pellets, Skretting, Norway). The fish were fasted for 2 d prior to drug administration and were not fed the first two days in the experimental period. The experiment was approved by the Norwegian Food Safety Authority (ID 10178).

2.2. Administration of feed

The medicated feed used for the per os (p.o.) administration was Amber Neptun (Skretting) containing 2 g active florfenicol (4 g Aquaflor premix, Intervet/Schering-Plough Animal Health) per kg feed. To ease administration, the medicated pellets were diluted 1:1 with sterile water (Sigma) and homogenized in a GentleMACS Dissociator (Miltenyi Biotec) using gentleMACS C tubes (Miltenyi Biotec). The paste was easily administered to the fish via a silicone hose and a syringe. Prior to drug administration, the fish were weighed and the amount of feed administered corresponded to a dose of 10 mg florfenicol per kg fish.

2.3. Sampling

Prior to administration of florfenicol-feed, six fish were killed by a blow to the head and samples of plasma and muscle, head kidney and liver tissues were obtained. Blood was sampled from the caudal vein using a 1 ml syringe. Plasma was isolated by centrifugation of blood at approximately 2000 *g* for 10 min. After administration of feed, four groups of six fish were placed in individual tanks (15 l, with through flow) following treatment to ensure as accurate sampling as possible. These four groups were used for the first four samplings. The remaining fish were kept in a 500 l tank. Samples were taken at 3, 6, 12, 24, 72, 120, 168, 240 and 336 h post administration as described above. ($n = 6$) All samples were immediately frozen and stored at −20 °C until analysed.

2.4. Analyses of florfenicol and florfenicol-amine

Tissue samples were homogenized in a Fast Prep-24™5G benchtop homogenizer (M.P. Biomedicals) using Lysing matrix tubes prefilled with matrix S metal beads (M.P. Biomedicals). The homogenized tissue and plasma samples were spiked with internal standards (Florfenikol-d3 and Florfenikol amine-d3; Toronto Research Chemicals, Inc.) and extracted with a mixture of 49% ethyl acetate, 49% acetonitrile and 2% ammonia solution (25%) (Xie et al., 2013). The mixtures were vortex-mixed and centrifuged before the extracts were transferred to a new vial and concentrated under nitrogen flow at 40 °C. The residues were dissolved in water/methanol (80:20) and filtered through a 0.45 µm filter. Analysis was performed using an Agilent 1290 LC-system (Agilent Technologies) coupled to an Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies). The analytes were separated by a reverse phase Agilent stable bond C18-column (150 mm × 2.1 mm i.d., 1.8 µm particle size) (Agilent Technologies) using a 0.4 ml/min flow. The mobile phase was a mixture of methanol and 0.1% formic acid in water. Chromatography was performed utilising a stepwise gradient: 0–0.9 min, 2% methanol; 1.0–2.0 min, 98% methanol; 2.1–4.0 min, 2% methanol. The instrument was equipped with an electrospray ionization (ESI) source with polarity switching, operated in a negative mode for florfenicol and its internal standard, and positive mode for florfenicol amine and its internal standard. The following source conditions were used: gas temperature: 200 °C; gas flow: 6 l/min; nebulizer pressure: 35 psi; sheath gas temperature: 350 °C; sheath gas flow: 12 l/min; capillary voltage: 3600 V (positive mode) and 2500 V (negative mode); nozzle voltage: 0 V (positive mode) and 100 V (negative mode). The analytes were monitored using the following transitions: florfenicol, 355,8 *m/z* → 185,0 m/z (quantifier) and 355,8 m/z → 119,1 m/z (qualifier); florfenicol-d3, 359,0 m/z → 121,1 m/z; florfenicol amine, 248 m/z → 230 m/z (quantifier) and 248 m/z → 130,1 m/z (qualifier); florfenicol amine-d3, 251 m/z → 233,1 m/z. Procedural blank, matrix blank, matrix-matched calibration curve and controls were prepared for each series. The limit of quantification (LOQ) for florfenicol was determined to 2.0 ng/ml in plasma and 2.0 ng/g in tissue samples. For florfenicol amine, the LOQ varied from 10 to 20 ng/g in the tissues, while the LOQ varied from 2.0 to 4.0 ng/ml in plasma. The method was linear over the range studied for florfenicol amine (LOQ – 400 ng/g). Florfenicol was linear up to 3000 ng/g; samples with levels above 3000 ng/g were diluted in order to establish a linear calibration curve. Recovery ranged from 90% to 110%, and relative standard deviation was < 15%.

2.5. Pharmacokinetic analysis

Standard pharmacokinetic parameters were calculated using the computer program PCNONLIN version 4.2 (Statistical Consultants Inc.). The best fitted models were chosen using Akaike's information criterion estimation (Yamaoka et al., 1978).

2.6. Bacterial culture

Twenty-eight isolates of *A. salmonicida* isolated from diseased lumpfish from different locations in Norway were cultured in tryptic soy broth (TSB) at 20 °C, 200 rpm until late log phase. The number of cells were determined using the cell counter CASY Modell TT 150 µm (Roche Diagnostics) and diluted to a concentration of 5×10^6 bacteria/ml.

2.7. Minimum inhibitory concentration (MIC) determinations

The MIC determinations were performed using microtest plate with 96-well with rounded bottom (Sarstedt AG & Co.). A two-fold dilution of florfenicol (Sigma) in the range of 0.0001–100 µg/ml were performed. Three parallels were performed for each concentration. Hundred µl of bacterial suspension (5×10^6 bacteria/ml) were mixed with 100 µl of antibacterial agents diluted in Tryptone Soya Broth (TSB). Negative controls containing bacterial suspension, but no antibacterial agents were included for each isolate. The plates were incubated at 20 °C for 48 h. The MICs were determined after visual inspection, and given as the concentration where no growth could be observed.

2.8. Statistical analysis

Analysis of variance (ANOVA) was performed to evaluate the effect of time using the statistical software package SigmaStat 3.5. Since the variance was not normally distributed, the *P* value cut off was set to 0.01 as suggested by Glass et al. (1972). The Holm-Sidak method was performed for pairwise multiple comparison.

3. Results

The mean florfenicol concentrations versus time in plasma, muscle, liver and head-kidney tissues are given in Table 1 and Fig. 1. The highest concentration of florfenicol was detected 24 h post administration in plasma and all the tissues. The metabolite florfenicol amine was found in plasma and all examined organs, although in small amounts, with the highest concentrations after 24 h (Fig. 2). The highest concentration of florfenicol amine, 0.21 µg/g, was found in head-kidney after 24 h (Fig. 2B). The 24 h time point was significantly higher than the other time points, except for 12 h post oral administration (Table 3). For all samples, both the florfenicol and florfenicol-amine samples, there was a significant effect of time ($P < .001$) (Table 3). Pharmacokinetically, the florfenicol plasma data was best described by a one-compartment open model with first-order input, first-order output and no lag-time. The values from Table 1 were used to calculate the pharmacokinetic parameters in PCNONLIN. The peak plasma concentration (C_{max}) was calculated to be 3.55 µg/ml, the time to peak plasma concentration (T_{max}) to be 21.2 h and the elimination halflife ($t_{½}$ β) to be 30 h. Area under curve (AUC) and AUC from 0 to 24 h ($AUC_{0–24h}$) were calculated to be 248 and 61 h µg/ml, respectively. The pharmacokinetic parameters for plasma, muscle, head kidney and liver are given in Table 2 and the statistical analyses are summarized in Table 3.

The susceptibility of florfenicol against 28 isolates of *Aeromonas salmonicida* isolated from diseased lumpfish ranged from 0.39 to 1.56 µg/ml and where three isolates had MIC value of 0.39 µg/ml, 14 isolates MIC value 0.78 µg/ml and 11 isolates MIC value 1.56 µg/ml.

Table 1
Florfenicol (FF) and florfenicol-amine (FFA) concentration in plasma (µg/ml) and tissues (µg/g) at different time points post oral administration of 10 mg/kg.

	Sample	Time (h)										
		0	3	6	12	24	72	96	168	240	336	
FF	Plasma	< LOQ[a]	1.508 ± 0.854	2.086 ± 1.017	2.915 ± 0.507	3.519 ± 0.885	1.555 ± 0.735	0.399 ± 0.074	0.129 ± 0.107	0.034 ± 0.022	0.003 ± 0.003	
	Head kidney	< LOQ[a]	2.150 ± 1.611	2.882 ± 1.467	4.260 ± 1.338	4.622 ± 1.798	2.027 ± 0.740	0.639 ± 0.115	0.243 ± 0.175	0.073 ± 0.052	0.004 ± 0.007	
	Lever	< LOQ[a]	1.273 ± 0.809	1.947 ± 1.178	2.175 ± 0.908	2.543 ± 1.012	0.965 ± 0.437	0.283 ± 0.081	0.1 ± 0.049	0.026 ± 0.037	0.004 ± 0.008	
	Muscle	< LOQ[a]	1.173 ± 0.906	1.772 ± 1.055	2.337 ± 1.073	3.108 ± 1.372	1.114 ± 0.434	0.333 ± 0.070	0.127 ± 0.089	0.030 ± 0.028	< LOQ[a]	
FFA	Plasma	< LOQ[a]	0.003 ± 0.004	0.007 ± 0.005	0.011 ± 0.004	0.021 ± 0.008	0.014 ± 0.009	< LOQ[a]	< LOQ[a]	< LOQ[a]	< LOQ[a]	
	Head kidney	< LOQ[a]	0.029 ± 0.029	0.051 ± 0.030	0.139 ± 0.059	0.211 ± 0.127	0.089 ± 0.047	0.036 ± 0.019	0.010 ± 0.011	< LOQ[a]	< LOQ[a]	
	Lever	< LOQ[a]	0.045 ± 0.038	0.061 ± 0.041	0.077 ± 0.036	0.134 ± 0.063	0.090 ± 0.066	0.013 ± 0.015	0.004 ± 0.009	< LOQ[a]	< LOQ[a]	
	Muscle	< LOQ[a]	0.007 ± 0.011	0.022 ± 0.022	0.036 ± 0.033	0.076 ± 0.027	0.043 ± 0.025	0.009 ± 0.007	< LOQ[a]	< LOQ[a]	< LOQ[a]	

[a] LOQ = limit of quantification.

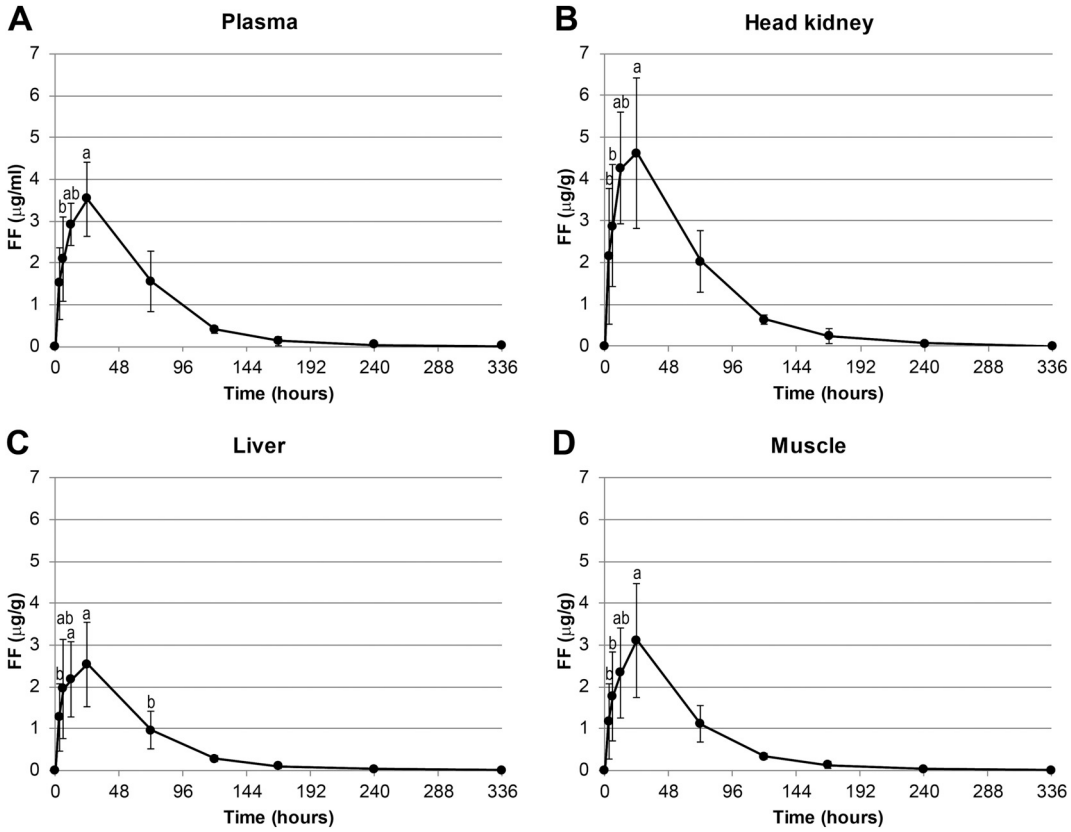

Fig. 1. Diagrams of uptake and elimination of florfenicol (FF)) at different time point post oral administration of medical feed (10 mg/kg). Concentrations of FF in plasma (A), head kidney (B), liver (C) and muscle (D). Time points are significant different statistically f they do not shear letter. Full statistical analysis is shown in Table 3.

4. Discussion

Knowledge of pharmacokinetics in lumpfish is important. The number of prescriptions of antibacterials to lumpfish is increasing due to infection problems, despite the lack of knowledge regarding pharmacokinetics and efficient treatment regimes for diseased lumpfish. A number of studies have been published describing the pharmacokinetics of florfenicol in fish, but only a few (Martinsen et al., 1993; Horsberg et al., 1996; Samuelsen et al., 2003; Lim et al., 2010; de Ocenda et al., 2017) were conducted under conditions comparable to the present study with regard to external parameters such as salinity and water temperature. A one-compartment open model with first-order input, first-order output and no lag-time best described the plasma data following a single oral administration. This is a pharmacokinetic model often used to describe a single oral administration of an antibacterial agent in fish and has been applied for orally administered florfenicol in Atlantic cod, Atlantic salmon and turbot (Martinsen et al., 1993; Horsberg et al., 1996; Samuelsen et al., 2003; de Ocenda et al., 2017).

Using T_{max} as indicator, our results show that florfenicol is rather slowly absorbed in lumpfish compared to other fish species. While olive flounder (*Paralichthys olivaceus* Temminck & Schlegel, 1846), Atlantic salmon, Atlantic cod and turbot have plasma T_{max} values from 4 to 13.8 h, respectively, the plasma T_{max} of lumpfish is 21.2 h (Martinsen et al., 1993; Horsberg et al., 1996; Samuelsen et al., 2003; Lim et al., 2010; de Ocenda et al., 2017). Following a single oral administration of 10 mg/kg florfenicol to Atlantic salmon, C_{max} was calculated to 9.1 and 4.0 µg/ml respectively, by Horsberg et al. (1996) and Martinsen et al. (1993). In Atlantic cod, a similar dose gave C_{max} of 10.8 µg/ml (Samuelsen et al., 2003) whereas a single dose of 20 mg/kg gave C_{max} of 12.81 in olive flounder (Lim et al., 2010). The C_{max} of lumpfish with 3.55 µg/ml is therefore considerably lower than in Atlantic cod and in Atlantic salmon if compared with the results in Horsberg et al. (1996). In this study, it was shown that T_{max} and C_{max} values differed between lumpfish and other fish species. As lumpfish prefers to attach to the substrate using their ventrally located suction disk rather than swim actively, the physiological processes in this species may be slower, and thereby count for the differences. It is known that lumpfish excrete low levels of cortisol upon stress compared with cod, salmon and wrasse (Iversen et al., 2014) and has a different response to stress and external stimuli compared with salmon and zebrafish (Hale, 2000; Skår et al., 2017).

Plasma elimination half-lives ($t_{1/2}\beta$) of florfenicol vary significantly in marine fish. Compared with Atlantic salmon ($t_{1/2}\beta$ of 14.7 and 12.2 h, t = 10 °C), the elimination in lumpfish with $t_{1/2}\beta$ of 30 h at a temperature of 12 °C can be characterized as slow (Martinsen et al., 1993; Horsberg et al., 1996; Ocenda et al., 2017). However, in both Atlantic cod and olive flounder, florfenicol is eliminated at an even slower rate with $t_{1/2}\beta$ values of 39 h (t = 8 °C) and 49 h (t = 18.5 °C), respectively (Samuelsen et al., 2003; Lim et al., 2010). In lumpfish, there is a similarity in elimination between plasma and tissues ranging from 24 h in muscle to 33 in head kidney. In Atlantic cod, on the other hand, a much larger difference is found in $t_{1/2}\beta$ values between plasma ($t_{1/2}\beta$ = 39 h), muscle ($t_{1/2}\beta$ = 21 h) and liver ($t_{1/2}\beta$ = 20 h).

In this study plasma AUC and AUC_{0-24h} were calculated to be 248 and 61 h µg/ml respectively. Previous publications report only AUC values and compared to Atlantic salmon with AUC of 140 and 112 h µg/ml (Martinsen et al., 1993; Horsberg et al., 1996), the AUC of lumpfish is approximately twice as large. The AUC in Atlantic cod was calculated to 524 h µg/ml which is twice that found in lumpfish (Samuelsen et al., 2003). This difference is due, inter alia, to variance in elimination rates between the species. Based on tissue analysis and the calculated

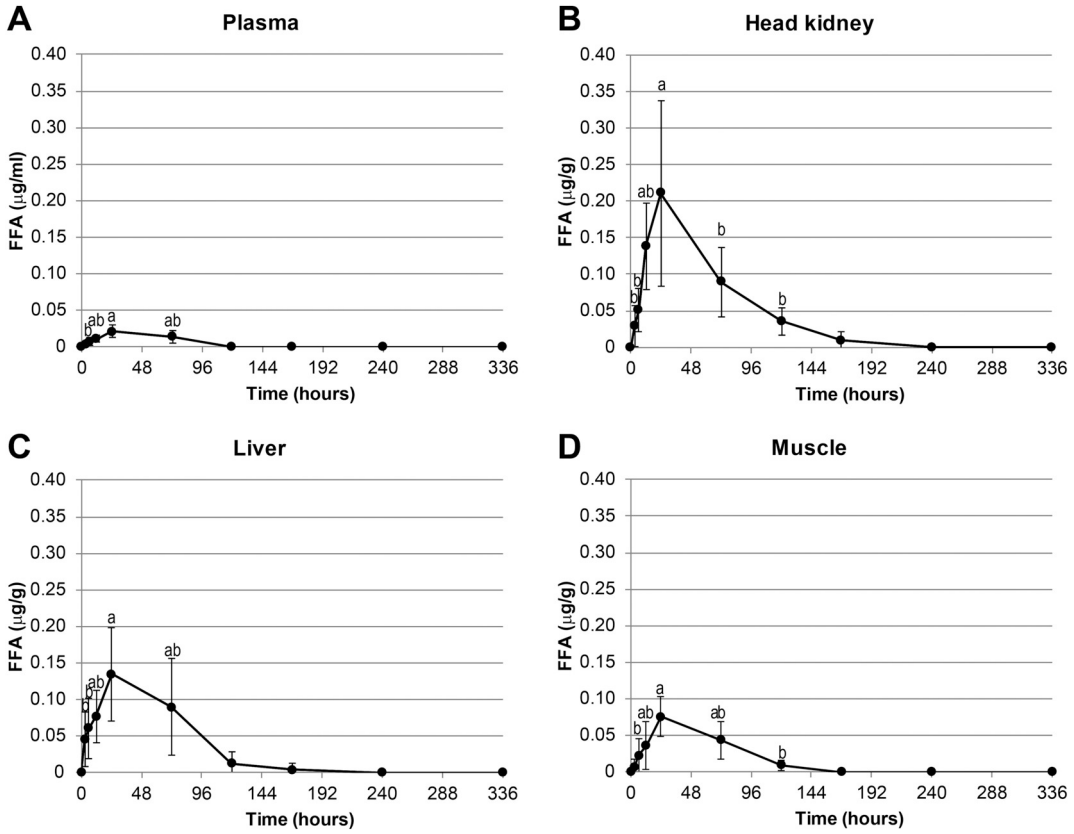

Fig. 2. Diagrams of uptake and elimination of Florfenicol amine (FFA) at different time point post oral administration of medical feed (10 mg/kg). Concentrations of FFA in plasma (A), head kidney (B), liver (C) and muscle (D). Time points are significant different statistically f they do not shear letter. Full statistical analysis is shown in Table 3.

pharmacokinetic values shown in Tables 1 and 2 and in Figs. 1 and 2, it can be concluded that florfenicol is well distributed throughout the body of lumpfish. Head kidney display the highest concentrations, followed by muscle and liver.

Florfenicol amine is described as a main metabolite of florfenicol in Atlantic salmon and was found in higher concentrations than florfenicol in plasma 48 h after the first administration in a multiple-dose study (10 mg/kg day for 10 consecutive days) (Horsberg et al., 1996). Following a single oral administration of 20 mg/kg of florfenicol to the fresh water fishes rice field eel (*Monopterus albus*) and Korean catfish (*Silurus asotus*), ratios of approximately 4:1 and 3:1 between florfenicol and florfenicol amine were found at T_{max} (Park et al., 2006; Xie et al., 2013). In Atlantic cod and olive flounder, however, florfenicol amine was not detected in quantifiable amounts in either plasma or tissues (Samuelsen et al., 2003; Lim et al., 2010). In this investigation, we found florfenicol amine in low concentrations, indicating that this specific metabolic pathway is of minor importance in lumpfish. A central application of pharmacokinetic and pharmacodynamic data is to establish appropriate treatment regimens which optimise efficacy and minimize the opportunity for the development of antimicrobial resistance.

Traditionally, the clinical significance of pharmacokinetic data was related to an assumption that the in vivo plasma concentration of the agent should exceed its minimum inhibitory concentration (MIC) value for the relevant pathogen by a factor of 3–4 (Stamm, 1989). Later, Shojaee AliAbadi and Lees (2000) suggested that an optimal dosage regimen should maintain concentrations at the site of infection in excess of MIC_{90} for the entire medication period for bacteriostatic drugs and bactericidal drugs acting primarily by time-dependant mechanisms while an Area Under $Curve_{0–24}$ ($AUC_{0–24}$)/MIC ratio of at least 100 and a peak concentration C_{max}/MIC ratio of at least 8 should be provided for bactericidal agents acting mainly by concentration-dependant mechanisms (known as PK/PD indices). While the magnitude of PK/PD indices required for efficacy has been studied in humans and terrestrial animals no studies of which PK/PD indices to use for the two most used antibacterials in Norwegian aquaculture, florfenicol and oxolinic acid, are available (Nightingale et al., 2007). It is appropriate, therefore, to evaluate efficacy using all three PK/PD indices. In lumpfish, the relevant pharmacokinetic values are C_{max} of 3.55 µg/ml and $AUC_{0–24}$ of 61 h µg/ml. The three MIC values of 0.39, 0.78 and 1.56 µg/ml give

Table 2
Calculated pharmacokinetic parameters for florfenicol in plasma, muscle, head kidney and liver of lumpfish following a single oral administration of 10 mg/kg.

Tissue	AUC $AUC_{0–24}$	C_{max}	T_{max}	$T_{1/2}\beta$	
Plasma	248 ± 14 h µg/ml	61 h µg/ml	3.55 ± 0.11 µg/ml	21.2 ± 1.3 h	30 ± 4 h
Muscle	197 ± 14 h µg/g	26 h µg/g	2.99 ± 0.12 µg/g	22.1 ± 1.7 h	24 ± 6 h
Liver	172 ± 14 h µg/g	48 h µg/g	2.54 ± 0.10 µg/g	26.4 ± 1.3 h	33 ± 5 h
Head kidney	338 ± 14 h µg/g	84 h µg/g	4.70 ± 0.11 µg/g	19.4 ± 0.9 h	33 ± 3 h

AUC = Area Under Curve, $AUC_{0–24}$ = Area Under Curve from 0 to 24 h, C_{max} = maximum concentration, T_{max} = time to maximum concentration, $t_{1/2}\beta$ = elimination half-life.

Table 3
Statistical analyses of the pharmacokinetical data of florfenicol (FF) shown in Fig. 1 and florfenicol-amine (FFA) shown in Fig. 2.*

Sample		Sampling time points following oral administration (hours)										Significance
		0	3	6	12	24	72	120	168	240	336	
FF	Plasma	d	c	bc	ab	a	c	d	d	d	d	$F_{9,56}$ = 27.007 (P < .001)
	Head kidney	e	bd	bc	ac	a	cd	de	e	e	e	$F_{9,56}$ = 20.044 (P < .001)
	Liver	e	bcd	ab	ac	a	bce	de	e	e	e	$F_{9,56}$ = 14.176 (P < .001)
	Muscle	c	bc	b	ab	a	bc	c	c	c	c	$F_{9,56}$ = 14.104 (P < .001)
FFA	Plasma	cd	d	b	abc	a	ab	cd	d	d	d	$F_{9,56}$ = 15.512 (P < .001)
	Head kidney	cdef	bfh	bd	ab	a	bc	beg	cdgh	cdef	cdef	$F_{9,56}$ = 11.719 (P < .001)
	Liver	c	bc	bc	abc	a	ab	c	c	c	c	$F_{9,56}$ = 09.800 (P < .001)
	Muscle	c	cd	bc	ab	a	abc	bd	c	c	c	$F_{9,56}$ = 13.934 (P < .001)

* Time points are significant different statistically if they do not shear letter.

C_{max}/MIC values of 9, 4.5 and 2.3 respectively. Therefore, if the suggestions by Shojaee AliAbadi and Lees (2000) are applied, florfenicol will, at a dosage of 10 mg/kg only give sufficient effect if the fish is infected by the most sensitive isolates. Corresponding results were obtained using the PK/PD indices AUC_{0-24}/MIC that gave values of 156, 78 and 39 h, respectively. Using the "time above MIC" (T > MIC) as the PK/PD indices, our investigation shows that florfenicol reached a C_{max} of 3.55 μg/ml and is slowly eliminated with a $t_{1/2}\beta$ of 30 h. This indicates that if florfenicol is administered once a day the plasma concentration will exceed the MIC's for the entire medication period for all three groups of isolates.

5. Conclusion

Our results indicate that an oral administration of 10 mg/kg of florfenicol will be effective in treating *A. salmonicida* infection in lumpfish, at least if caused by the most sensitive strains. The data generated here is important for development of efficient protocols for antibacterial treatment, although dose response studies will be needed for verification of the results and to propose a final recommendation of dose. Also, the data make the basis for calculation of withdrawal time and for reducing the risk for development of antimicrobial resistant bacteria.

Statement of relevance

1) In recent years, there has been an increasing number of prescriptions of antibacterials to lumpfish despite lack of knowledge regarding effective treatment regimes.
2) Knowledge of pharmacokinetics combined with determination of antibiotic resistance among fish pathogenic bacteria and effect of antibacterial agents on diseased lumpfish is important for the welfare of lumpfish. Additionally, it reduces the risk of development of drug-resistant bacteria.

Acknowledgements

We would like to thank to Felicia Dawn Couillard for contributing to the analysis of florfenicol and florfenicol amine. We thank Anita Rønneseth for giving one of the isolates used in the MIC analysis and Mark Powell for valuable discussion about the statistics. Funding: The Norwegian Seafood Research Fund (project 901468, HIW) and Research Council of Norway (Grant 244148, GTH).

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