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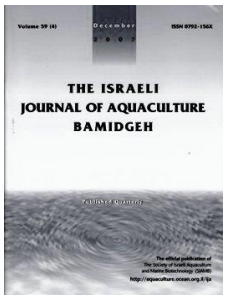

Pharmacokinetic Profile and Muscle Residue Elimination of Tilmicosin after Oral Administration in Crucian Carp (*Carassius auratus*)

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Abstract

The aim of this study was to investigate the pharmacokinetics and muscle tissue residue elimination of tilmicosin (TLM) in crucian carp (*Carassius auratus*) at water temperature of 26±1°C after administration of TLM at three different doses. The crucian carp were randomly divided into two treatment groups and a control group. TLM was administered at two different doses to the treatment groups. Group 1 received a single orally administered dose of TLM at the rate of 50mg/kg body weight, group 2 received three orally administered doses of TLM at the rate of 50 mg/kg body wt. on three consecutive days and Group 3 (the control group) received a normal, drug free diet (0 TLM).

TLM concentrations in plasma and muscle tissue were analyzed with ultra high-performance liquid chromatography (UPLC, Waters, USA). Using the 3p97 software, the data of pharmacokinetics was conformed to a two-compartment model. The absorption rate constant (*K*a) and absorption half-life ($t_{1/2\ k\alpha}$) of TLM were 1.99 h and 0.349 h, respectively. The distribution half-life ($t_{1/2\alpha}$) and elimination half-life ($t_{1/2\beta}$) of TLM were 2.87 h and 39.89 h, respectively. The maximum concentration (C_{max}) of TLM in plasma was 19.36 μg/ml and the time to peak concentration (T_p) was 1.27 h. The area under the plasma concentration-time curve (AUC) was 240.326 μg·h/ml. The distribution volume (V_d/F) of TLM was calculated as 1.968 l/kg. for determination of the muscle residue elimination of TLM used by oral administration at a dosage of 50 mg/kg body weight per day for 3 days of TLM. The results revealed that the elimination of TLM in the crucian carp muscle tissue has a long half-life time of 3.12 d. Based on the above residues, we suggest the withdrawal time should be at least 33 days, calculated by the equation $MRL=C_0\ e^{-k(WDT)}$.

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Introduction

Crucian carp (*Carassius auratus*) is one of the main fresh-water cultured species in China. Production is approximately 250 thousand metric tons per year however crucian carp is easily infected by bacteria and suffers from enteritis, gill-rot, and red skin disease, etc. In order to ensure high quality crucian carp, prevention and treatment of these diseases is imperative. Macrolides, a group of antibacterial compounds are effective against gram-positive and some gram-negative bacteria and are now widely used in human and veterinary medicines.

Tilmicosin (TLM), derived from tylosin, is a type of antimicrobial macrolide. Its activity against *Pasteurella multocida* and *P. hemolytica* is greater than that of tylosin (Debono et al., 1989; Rérat et al., 2012). Lower resistance of TLM was observed compared with tiamulin, ampicillin and tetracycline used against *A. pleuropneumoniae* (Kucerova et al., 2011). TLM is widely used in veterinary medicine to treat respiratory diseases of cattle, swine and chicken, etc. TLM and derivatives have also been used for treating gram-positive bacteria which cause diseases of aquatic species (Eli Lilly and Company, Lily Corporate Center, 1994). 40.4% of six gram-negative bacterial pathogens (*Edwardsiella ictaluri*, *Edwardsiella tarda*. *Aeromonas* sp., *Aeromonas hydrophila*, *Aeromonas sobria* and *Pseudomonas* sp.) of channel catfish were susceptible to TLM (Plumb et al., 1995). Diets containing TLM at 200, 600, and 1000 mg/kg were palatable to Atlantic salmon and were consumed at the same rate as the control feed. Treated fish grew as efficiently as the control fish, and post-mortem examination of all of the fish at the end of the study revealed no gross abnormalities (Eli Lilly and Company Lily Corporate Center, 1994). There have been many studies on pharmacokinetics and residue depletion of TLM in mammals (Ramadan, 1997; Ziv et al., 1995; Parker et al., 1994a; Parker et al., 1994b; Shen et al., 2005; Zhang et al., 2004). The EU has set the Maximum Residue Limits for TLM in animal products. The MRL in fin fish is 50 μg/kg (EU, 2004), and the same MRL value was established in salmoniformes, anguilliformes, perciformes, and other fish, shelled molluscs, crustaceans and other aquatic animals (the Japanese positive list, 2013). However, the pharmacokinetics and residue elimination of TLM in aquatic animals is unknown.

The aim of this study is to determine the pharmacokinetic parameters and the elimination time of TLM in crucian carp at water temperature of 26 ±1 °C.

Materials and methods

Chemicals: Standard TLM was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Ethyl acetate, acetonitrile, methanol, acetic ether, hexane of HPLC grade were purchased from J.T. Baker (Deventer, Holland). The water used in this study was prepared with a Milli-Q Gradient A10 water system (Millipore, Bedford, MA, USA). Ammonium dihydrogen phosphate, anhydrous sodium sulfate and sodium dihydrogen phosphate were analytically pure and purchased from Sinopharm Chemical Reagent company (Shanghai, China).

TLM standard stock solution, (100 μg/ml), was prepared by dissolving TLM in methanol and stored at -20 °C. The TLM working solutions were prepared by diluting the stock solution in 25 mM ammonium dihydrogen phosphate buffer mixed with acetonitrile 70:30 v/v.

Experimental fish: 230 healthy crucian carp (*C. auratus)*, mean body weight, 200±18 g) were provided by Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Science, Wuhan China. The fish were divided into three groups: (1) for pharmacokinetic research, (2) for residue assessment ,(3) the control group. Fish were placed in fiberglass tanks supplied with 300 l dechlorinated tap water. Oxygen content was kept close to saturation with air-stones. The fish were fed with drug-free normal feed for 7 days, then starved for another day before the beginning of the drug administration test. During the entire experimental period the fish were raised under a photoperiod of 16 hours light and 8 hours darkness. Water temperature was maintained at 26±1 °C.

Drug administration: The commercial TLM technical powder (≥98% tilmicosin) was purchased from Tairui Pharmaceutical company (Ningxia, China), and was dissolved in a small amount of ethanol and diluted with distilled water. The solution of 10 mg/ml TLM was obtained for oral administration.

For the study of pharmacokinetics, the fish were given a single dose of 50 mg/kg body weight TLM suspension. For the study of residues the fish were given 50 mg/kg body weight for 3 days. The control group was given drug-free feed.

Preparation Sampling: About 2 ml blood was taken from the caudal vein of 5 randomly chosen fish at every sampling, ranging from 10 min to 168 h after oral administration. 1% heparin was used to prevent clotting. Plasma was isolated by centrifugation at 3500 r/min for 5 min and stored at -20°C for the pharmacokinetic research.

For the residue study, muscle was sampled on 1, 2, 3, 5, 7, 9, 12, 15, 20, 25, and 30 days after administration. The muscle tissue of five fish was collected at each sampling from the experimental groups and the control group. The samples were immediately frozen and stored at -20 °C until use.

Analyses of samples: The analysis of TLM in the plasma and muscle were performed on a UPLC system (Waters, USA), consisting of a binary solvent manager, sample manager, column heater and TUV detector at 287 nm. The chromatographic column was a 100 mm×2.1 mm i.d. stainless steel column packed with 1.7 μm particle size reverse-phase chromatography medium (ACQUTY UPLC BEH C18, Waters). The column temperature was maintained at 40 °C. The mobile phase consisted of 25 mM ammonium dihydrogen phosphate buffer with 10% acetonitrile as eluent A and pure acetonitrile as eluent B at the flow rate of 0.3 ml/min. The gradient is listed in Table 1. Data acquisition and integration were performed using Empower 2 chromatographic management software (Waters, USA).

Table 1**.** UPLC gradient profit for determination of TLM

Time (min)	*Flow rate (ml/min)*	*Composition of mobile phase*		Curve
		Eluent A (%)	*Eluent B (%)*	
0	0.3	10	90	Initial
1.5	0.3	10	90	6
6	0.3	30	70	6
6.01	0.3	10	90	1
7	0.3	10	90	6

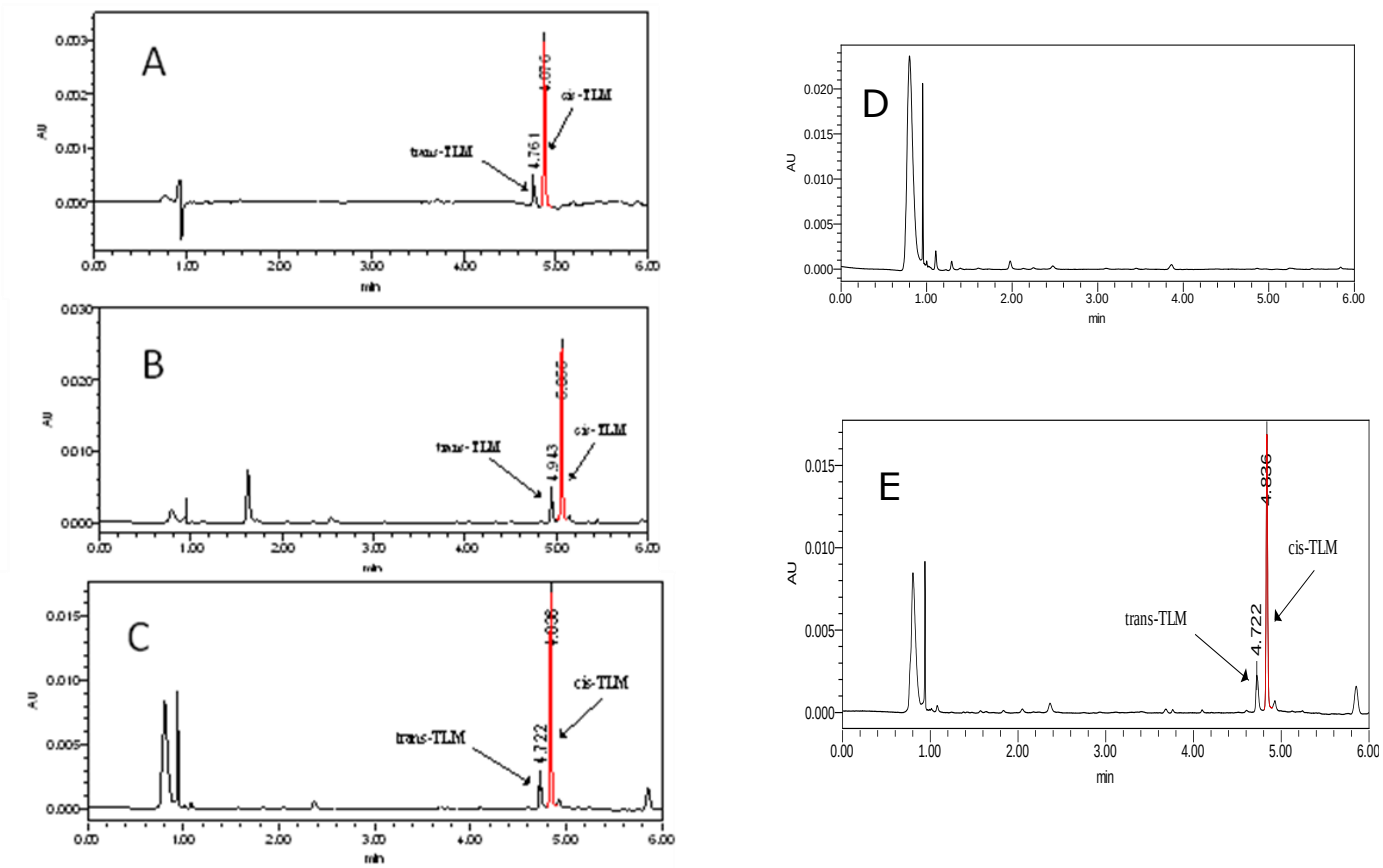
The plasma samples were thawed at room temperature. A sample of 1 ml plasma was transferred to a 10 ml plastic centrifuge tube with a screw cap. About 1 ml of 50 mM sodium dihydrogen phosphate water solution was added to precipitate proteins in the sample and to adjust pH value. Each sample was mixed in a Whirlimix vortex mixer for 30 s, and 4 ml of ethyl acetate was added to each tube, and shaken vigorously for 30 s. Samples were then centrifuged at 5000 rpm for 5 min. The supernatant was removed and transferred to another 10 ml plastic centrifuge tube with a screw cap. The residue was re-extracted with 2 ml of ethyl acetate. All the organic phases were combined and dried under a gentle stream of nitrogen at 40 °C. The residue was dissolved in 1 ml of 25 mM ammonium dihydrogen phosphate buffer mixed with acetonitrile 70:30, v/v. After brief mixing in a vortex mixer, 2 ml hexane was added and the mixture was mixed in a vortex mixer for 30 s. After centrifugation at 5000 rpm for 5min, the hexane layer was removed. The water-based phase was filtered through a 0.22 μm membrane filter, and 7 μl was injected into the UPLC system.

2 g of homogenized muscle was transferred to a 50 ml plastic centrifuge tube with a screw cap. 10 ml of acetonitrile was added and the sample was mixed in the vortex mixer for 30 s. 2 g of anhydrous sodium sulfate was added to the sample in order to remove water and precipitate proteins. The sample was shaken vigorously for 30 s and centrifuged at 5000 rpm for 5 min, and the supernatant transferred to a 150 ml pear shaped rotovap flask.

The acetonitrile extraction procedure described above was repeated, and the consecutive acetonitrile extracts were placed in the same flask. The extracts were rotary evaporated in a water bath set at 45 °C and vacuum set 550 mbar to dryness. 1 ml of 50 mM sodium dihydrogen phosphate water solution was added in the flask, and the sample

was mixed in a Whirlimix vortex mixer for 30 s. 4 ml of ethyl acetate was added to the sample and mixed for 30 s. The sample was transferred from the flask to a 10 ml plastic centrifuge tube with a screw cap and centrifuged at 8000 rpm for 5 min. The supernatant was then transferred to another 10 ml plastic centrifuge tube with a screw cap. The ethyl acetate extraction procedure described above was repeated. The organic phase was combined and dried under a gentle stream of nitrogen at 40 °C. The residue was reconstituted in 1 ml of 25 mM ammonium dihydrogen phosphate buffer mixed with acetonitrile at 70:30 v/v, and then mixed in a vortex mixer for 30 s. The sample was centrifuged at 10,000 rpm for 5 min and filtered through a 0.22 µm membrane filter, and 7 µl was injected into the UPLC system. Typical UPLC chromatograms for TLM standards and extracts of control and actual samples are presented in Figure 1.

Fig 1. Chromatograms of TLM standard (0.1 µg/ml) (A); control crucian carp plasma (B); 120 h crucian carp plasma sample (C); control crucian carp muscle (D); 15 d crucian carp muscle sample (E). Peaks: trans-TLM and cis-TLM.



Method validation: The method was validated for plasma and muscle tissue. The standard calibration curve within the concentration of 0.025, 0.05, 0.1, 0.25, 0.5, 1, 5, 10, 20 µg/ml was used. The standard calibration curve was drawn with the sum of cis-TLM and trans-TLM peak areas as in the *Y* axis, and TLM concentrations as in the *X* axis. Recovery, intra- and inter-assay precision of the method were evaluated at concentrations of 0.1, 1.0, 10.0 µg/ml TLM (plasma) and 0.1, 1.0, 10.0 µg/g TLM (muscle). Recovery was determined by comparing the peak area from spiked samples with that of directly injected standard solutions. The intra- and inter-day precision was examined by analyzing five replicates of spiking samples on the same day and five consecutive days, respectively. The precision was expressed as the percentage relative standard deviation (%RSD). The limit of detection (LOD) and the limit of quantitation (LOQ) of TLM were defined as the concentrations of TLM resulting in a signal-to-noise ratio (S/N) of 3 and 10, respectively.

Pharmacokinetic and residue analysis: All processes were assumed to follow first-order kinetics. Pharmacokinetic parameters were calculated by the computer program 3P97 (version 1.0, edited by The Chinese Society of Mathematical Pharmacology, P.R. China). The model was selected based on the residual sum of squares and the minimum Akaike's information criterion (AIC) (Yamaoka and Nakagawa, 1978). The area under the

concentration-time curve (AUC) was calculated using the trapezoidal rule and was extrapolated to infinity (Ritschel, 1986). The volume of distribution (V_d/F), total body clearance (CL_b), the elimination half-life ($t_{1/2\beta}$ or $t_{1/2ke}$) and distribution half-life ($t_{1/2\alpha}$) were computed.

In the residue study, the elimination rate constant (K) was obtained from the slope of elimination curve on log-transferred TLM concentration (ln$C$) against time, and the elimination half-life ($T_{1/2}$) was calculated from $T_{1/2}$=ln2/k for muscle (Ding et al., 2006). The withdrawal period can be calculated when the tolerance limit of the concentration of all tissue samples is below the established maximum residue limit (MRL) (Tyrpenou et al., 2003).

Results

Method validation: Recovery of TLM was 90.2%-98.5% for plasma and 81.2%-90.5% for muscle, respectively. Intra-day and inter-day precisions were 2.14%-4.52% and 2.65%-5.04% for plasma, 3.05%-6.32% and 3.62%-8.47% for muscle. The detection limits for plasma and muscle were 0.010 μg/ml and 0.025 μg/g, respectively. The quantification limits for plasma and muscle were 0.025 μg/ml and 0.050 μg/g respectively. The standard curve of TLM showed Y=6392.2X-4556 in the range of 0.025 to 20.0 μg/ml, and the correlation coefficients (r^2) was 0.9995. The TLM concentrations in unknown plasma and muscle samples were calculated using the standard curve.

Pharmacokinetic analysis in plasma: The average concentrations of TLM versus time in plasma of crucian carp after administration of TLM are listed in Table 2.

Table 2 **.** TLM concentrations (μg/ml) in plasma of crucian carp (*Carassius auratus*) after oral administration at a single dose of 50 mg/kg body weight at water temperature of 26 ±1 °C (n=5)

Time (h)	Mean TLM concentrations±S.D (μg/ml)
0.167	11.016±2.028
0.25	11.655±0.999
0.5	13.974±0.915
1	20.781±3.534
2	18.530±2.355
3	14.578±3.110
5	10.824±1.808
6	6.829±1.888
8	6.255±1.247
10	5.326±0.935
12	3.632±0.213
16	3.001±0.661
18	1.492±0.675
24	1.424±0.677
48	0.954±0.326
96	0.779±0.059
120	0.564±0.095
144	0.479±0.105
168	0.097±0.053

The data obtained were best described by a two-compartment open model with a lag time using the equation: $C=Ae^{-\alpha(t-tL)}+Be^{-\beta(t-tL)}-(A+B)e^{-ka(t-tL)}$. Where C is the plasma concentration at any time (t), t_L is the lag time. A and B are zero-time intercepts of the curve best fitting the absorption and distribution of elimination phases, α and β are the distribution and elimination rate constants, K_a is the absorption rate constant. The TLM plasma levels (C)-time course was described by the equation in Figure 2: $C=26.040e^{-0.242(t-0.011)}+2.557e^{-0.0174(t-0.011)}-28.597e^{-1.986(t-0.011)}$.

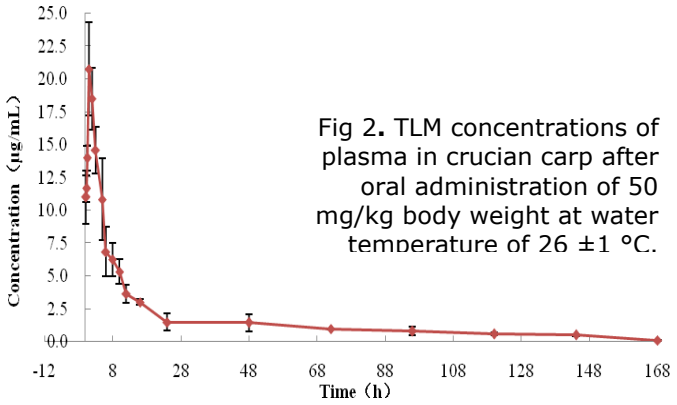


Fig 2**.** TLM concentrations of plasma in crucian carp after oral administration of 50 mg/kg body weight at water temperature of 26 ±1 °C.

The absorption rate constant (K_a) of TLM was 1.986/h, and the absorption, distribution, and elimination half-life ($t_{1/2a}$, $t_{1/2\beta}$, and $t_{1/2ka}$) were 2.865 h, 39.882 h and 0.349 h, respectively. The drug was well distributed to the tissues since the drug apparent volume of distribution was 1.968 l/kg. The maximum plasma concentration of TLM (C_{max}) and the time of reaching maximum plasma concentration (T_p) of TLM were 19.357 μg/ml and 1.269 h respectively. The area under

the concentration-time curve (AUC) was 240.326 μg·h/ml. The pharmacokinetic parameters are given in Table 3.

Table 3. Pharmacokinetic parameters for TLM in crucian carp (*C. auratus*) after oral administration at a single dose of 50 mg/kg body weight at water temperature 26 ±1 °C

parameters	*unit*	*value*
A	μg/ml	26.040
a	1/h	0.242
B	μg/ml	2.557
β	l/h	0.0174
K_a	1/h	1.986
t_L	h	0.011
V_d/F	l/kg	1.968
$t_{1/2a}$	h	2.865
$t_{1/2\beta}$	h	39.882
$t_{1/2Ka}$	h	0.349
K_{21}	1/h	0.0398
K_{10}	1/h	0.106
K_{12}	1/h	0.114
AUC	μg·h/ml	240.326
CL_b	(ml/h)/kg	0.208
T_p	h	1.269
C_{max}	μg/ml	19.357

A and *B* are zero time intercept of distribution phase and zero time intercept of elimination phase, respectively; *α:* distribution rate constant; *β:* elimination rate constant; K_a: absorption rate constant; t_L: Lag time; V_d/F: the volume of distribution; $t_{1/2\alpha}$: distribution half-life; $t_{1/2\beta}$: elimination half-life; $t_{1/2ka}$ absorption half-life; K_{12} and K_{21} : first-order rate constants for drug distribution between the central and peripheral compartment; CL_b: total body clearance; K_{10}: elimination rate constant from the central compartment; T_p: the time point of maximum plasma concentration of the drug; C_{max}: the maximum plasma concentration.

Residue elimination of TLM in crucian carp. The mean concentrations of TLM versus time in muscle tissue of crucian carp after multiple-dose oral administration were given in Table 4.

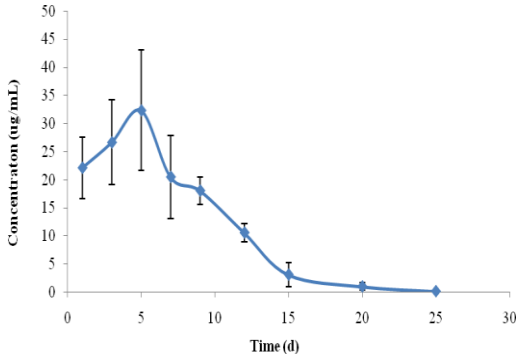
Table 4. The concentrations (μg/g) of TLM residues in crucian carp (*C. auratus*) muscle tissues (n=5)

Time (d)	*Mean TLM concentractions ±S.D.*
	muscle
1	22.12±5.50
3	26.65±7.51
5	32.34±10.67
7	20.49±7.42
9	18.05±2.39
12	10.57±1.65
15	3.09±2.17
20	0.98±0.67
25	0.13±0.095
30	ND

ND: not detected

Residue concentration of TLM was 22.12±5.50 μg/g on the first day, and TLM concentration reached the highest of 32.34±10.67 μg/g on the fifth day. Residue concentration was 0.13±0.095 μg/g in muscle on the 25th day, whereas TLM concentration was less than the detection limit (0.025 μg/g) on the 30th day. The elimination half-life ($T_{1/2}$) in muscle tissue was calculated to 3.12 d. The curve of muscle concentration versus time detected in the crucian carp after multiple-dose oral administration at water temperature of 26 ±1 °C were shown in Figure 3.

Fig 3. Elimination of TLM in muscle of crucian carp (*C. auratus*) after oral administration at a multidose of 50 mg/kg body weight for 3 days (one dose each day), water temperature of 26 ±1°C.



The MRL for TLM in fin fish was 50 µg/kg (EU., 2004). The withdrawal time (WDT) was calculated using the following equation: MRL=C_0 $e^{-k(\mathrm{WDT})}$ (Ding et al., 2006). The withdrawal period should not be less than 33 days at water temperature of 26±1 °C.

Discussion

The methods of determination of TLM in mammal plasma or serum (Bennett et al., 1966; Michael et al., 2007; Abu-Basha et al., 2007; Shen et al., 2005; Womble et al., 2006; Clark et al., 2008) and in fish muscle (Horie et al., 2003; Houda et al., 2008; Simth et al., 2009) include analytical techniques such as microbiological assay, liquid chromatography (LC) with ultraviolet (UV), LC-mass spectrometry and LC-tandem mass spectrometry (LC-MS/MS). We developed a method for the determination of TLM in plasma and muscle of crucian carp using ultra performance liquid chromatography (UPLC) with tunable ultraviolet (TUV). The UPLC method for the determination of TLM has some characteristics such as ultra high resolution, ultra high speed, high sensitivity compared with conventional HPLC. The cis- and trans-isomers of TLM were separated on the ACQUTY UPLC BEH C18 column. The limit of quantification (LOQ) for TLM in chicken plasma was 0.05 µg/ml (S/N=6), and analysis time was 10 min using HPLC with UV detection (Abu-Basha et al., 2007). The limit of quantification in bovine and porcine serum were both 0.1 µg/ml (S/N=6) by LC with UV detector, and the analysis time was 30 min. (Moran et al. 1997). However, the limit of quantification for TLM in crucian carp plasma was 0.025 µg/ml (S/N=10), and the analysis time was only 7 min using UPLC with a TUV detector. Recoveries were 91%-95% for bovine serum, 85%-93% for porcine serum, 87.1±3.3% for yellowtail muscle, 89.2±4.2% for red sea bream muscle, 87% for sea bream filets and 79% for trout filets, respectively (Moran et al., 1997; Masakazu et al., 2003; Houda et al., 2008). In this study, recoveries were 90.2%-98.5% for plasma and 81.2%-90.5% for muscle, respectively. In summary, we developed a rapid and accurate UPLC method for determination of TLM in crucian carp plasma and muscle.

In this study, a two-compartment open model may best describe TLM disposition in the crucian carp in water temperature of 26 ±1 °C. The same compartment model was used in swine (Shen et al. 2005), whereas Abu-Basha et al. (2007) and Modric et al. (1999) performed the pharmacokinetic analysis of the data using noncompartmental analysis. The distribution half-life ($t_{1/2\alpha}$) and elimination half-life ($t_{1/2\beta}$) of TLM in the crucian carp after oral administration at a single dose of 50 mg/kg body weight were 2.865 h and 39.882 h, respectively, close to the $t_{1/2\alpha}$ and $t_{1/2\beta}$ of TLM in swine after oral administration at dose of 40 mg/kg body weight were 3.20±0.76 h and 20.69±5.07 h, respectively. It was also reported that $t_{1/2\beta}$ of liquid TLM and powder TLM in chicken after administration of a single oral dose of 30 mg/kg body weight using noncompartmental analysis were 47.4±9.32 h and 45.0±5.73 h, respectively (Shen et al., 2005; Abu-Basha et al. 2007). Those results indicated that TLM is quickly absorbed, distributed and slowly eliminated after oral administration in crucian carp. An obvious difference was observed in the maximum plasma concentration (C_{max}) between the crucian carp, swine, and chicken. In this study, the C_{max} of TLM in crucian carp was 19.357 µg/ml, however, C_{max} of TLM were 1.19±0.30 µg/ml and 2.03±0.28 µg/ml in swine after oral administration of two doses of 20 mg/kg and 40 mg/kg body weight respectively (Shen et al., 2005). C_{max} of liquid TLM and powder TLM in chicken were 2.09±0.37 µg/ml and 2.12±0.40 µg/ml, respectively, after administration of a single oral dose of 30 mg/kg body weight (Abu-Basha et al., 2007). There appeared to be metabolic differences related to species differences. The elimination of TLM may also be related to body temperature. The body temperature of warm blooded animals remains at about 37 °C, and body temperature of fish is related to the water temperature of 0.5-1 °C higher than water temperature. In this experiment, the body temperature of crucian carp was about 27 °C. The results

showed that the elimination of TLM was slower in crucian carp than in swine. The deleption of difloxacin in crucian carp decreased with a long half-time, especially at lower water temperature (Ding et al. 2006). Temperature had significant effect on the elimination of difloxacin, and lower temperature resulted in lower reactions (Sun et al. 2013). C_{max} of TLM in *Mycoplasma pulmonis*-infected and noninfected rats were 294 μg/ml and 309 μg/ml, respectively, after a single subcutaneous dose of 20 mg/kg body weight using noncompartmental pharmacokinetic analysis of serum concentrations (Modric et al. 1999). The MICs of TLM for *Corynebacterium pyogenes* and *staphylococcus aureus* were 0.04 μg/ml and 0.78 μg/ml respectively (Watkins et al., 1997). The MICs of TLM for *Pasteurella multocida* and *Mannheimia haemolytica* were 3.125 and 6.25 μg/ml respectively (Ziv et al. 1995). The distribution of diameters (mm) of zones of inhibition was created by tilmicosin (15 μg/kg) on six bacteria (*Edwardsiella ictaluri*, *Edwardsiella tarda*. *Aeromonas* sp., *Aeromonas hydrophila*, *Aeromonas sobria* and *Pseudomonas* sp.) isolated from channel catfish, and was as follows: Resistant$<$13 mm, intermediately susceptible 13-14 mm, susceptible$\geqslant$15 mm. (Plumb et al. 1995). In vitro evaluation of tilmicosin against a dozen strains of *Renibacterium salmoninarum* was reported. The minimum inhibitory concentration (MIC) was from 0.25 μg/ml to 2 μg/ml (Eli Lilly and Company Lily Corporate Center, 1994). It has been suggested that a ration of 4:1 between the average tissue concentration (C_{max}) and MIC_{50} is often considered sufficient (Horsberg et al., 1996).

The distribution volume (V_d/F) of TLM was 1.968 l/kg. indicating TLM was well distributed throughout the body. The finding is similar to those reported for cows, sheep, goats, and swine (Ziv et al., 1995; Ramadan, 1997; Modric et al., 1998; Shen et al., 2005). This distribution characteristic showed that TLM could be used for treating abscesses and lesions that may be caused by pathogenic bacteria in skin and muscle (Ferguson, 1989).

In this study, TLM residues in the muscle of crucian carp were higher than the maximum residue limit (MRL) (50 μg/kg) on day 25 after administration, but fell below the method detection limit on the 30th day after oral administration at a dosage of 50 mg/kg body weight per day for 3 days at water temperature of 26 ±1 °C. Through the study, the withdrawal time (WDT) suggested was 33 days in crucian carp muscle, whereas the minimum withdrawal time of 9 days in chicken muscle (Zhang et al. 2004). Compared with other macrolides, the WDT of TLM was longer than erythromycin in rainbow trout, Nile tilapia and gilthead sea bream (Esposito et al., 2007; Minh et al., 2011; Salvo et al., 2013), azithromycin in juvenile fall Chinook salmon (Fairgrieve et al., 2005). In contrast, Minh et al. (2010) observed a greater decrease in erythromycin concentrations in giant freshwater prawn (*Macrobrachium rosenbergii*) than TLM in crucian carp (Minh et al., 2010). The results above indicated that TLM metabolism differed greatly between different species, and TLM withdrawal period from the muscle tissue of crucian carp was longer. The results seen in this study may improve aquaculture practices in the future.

Conclusion

This study demonstrates the pharmacokinetic and residue elimination process of TLM in crucian carp. The results show that absorption and distribution of TLM was quick in crucian carp, whereas the elimination of TLM in crucian carp muscle was slow. To ensure the safety of edible tissue for human consumption, it is suggested that WDT should not be less than 33 days after oral administration with a dose of 50 mg/kg body weight per day for 3 days at water temperature of 26 ±1 °C.

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References

Abu-Basha, E.A., Idkaidek, N.M. and A.F. Al-Shunnaq, 2007. Pharmacokinetics of tilmicosin (Provitil powder and Pulmotil liquid AC) oral formulations in chickens. *Vet. Res. Commun.*, 31(4):477-485.

Bennett, J.V., Brodie, J.L., Benner, E.J. and W.M.H.Kirby, 1966. Simplified, accurate method for antibiotic assay of clinical specimens. *Appl. Microbiol.*, 14(2):170-177.

Clark C., Dowling P.M., Ross S., Woodbury M. and J.O. Boison, 2008. Pharmacokinetics of tilmicosin in equine tissues and plasma. *J. Vet. Pharmacol. Ther.*, 31(1): 66-70.

Ding F.K., Cao J.Y., Ma L.B., Pan Q.S., Fang Z.P. and X.C. Lu, 2006. Pharmacokinetics and tissue residues of difloxacin in crucian carp (*Carassius auratus*) after oral administration. *Aquaculture*, 256(1-4):121-128.

Debono M., Willard K.E., Kirst H.A., Wind J. A., Crouse G.D., Tao E.V., Vicenzi J.T., Counter F.T., Ott J.L. and E.E. Ose, 1989. Synthesis and antimicrobial evaluation of 20- deoxo-20-(3,5-dimethylpiperidin-1-yl) desmycosin (tilmicosin, EL-870) and related cyclic amino derivatives. *J. Antibiot.*, 42(8):1253-1267.

Eli Lilly and Company Lily Corporate Center, Tilmicosin and derivatives for treating gram-positive diseases of aquatic species: EU, 0606747A1[P]. 1994-07-20.

Esposito A., Fabrizi L., Lucchetti Dario., Marvasi L., Coni E. and E. Guandalini, 2007, Orally administered erythromycin in rainbow trout (*Oncorhynchus mykiss*): Residues in edible tissues and withdrawal time. *Antimicrob. Agents Ch.*, 51(3):1043-1047.

EU., 2004. Consolidated version of the Annexes I to IV of Council Regulation no. 2377/90 Updates up to 29. 10. 2004, 5 pp.

Fairgrieve, W. T., Masada C. L., McAuley W. C., Peterson M. E., Myers M. S. and M.S. Strom, 2005, Accumulation and clearance of orally administered erythromycin and its derivative, azithromycin, in juvenile fall Chinook salmon Oncorhynchus tshawytscha. *Dis. Aquatic Organ.*. 64:99-106.

Ferguson H.W., 1989. *Systemic Pathology of Fish*. Iowa State University, Ames. 263 pp.

Horie, M., Takegami, H., Toya, K., Kikuchi, Y. and H. Nakazawa, 2003. Detemination of spiramycin and tilmicosin in meat and fish by LC/MS. *Shokuhin Eiseiqaku Zasshi*, 44(3):150-154.

Horsberg T.E., Hoff K.A. and R. Nordmo, 1996. Pharmacokinetics of florfenicol and its metabolite florfenicol amine in Atlantic salmon. *J. Aquat. Anim. Health*, 8(4):292-301.

Houda B., Francesc B., Guillermina F. and M.M. Rosa, 2008. Determination of macrolide antibiotics in meat and fish using pressurized liquid extraction and liquid chromatography mass spectrometry. *J. Chromatogr. A*, 1208(1-2):83-89.

Kucerova Z., Hradecka H., Nechvatalova K. and K. Nedbalcova, 2011. Antimicrobial susceptibility of *Actinobacillus pleuropneumoniae* isolates from clinical outbreaks of porcine respiratory diseases. *Vet. Microbiol.*, 150(1-2):203-206

Masakazu H., Harumi T., Kazuo T. and N. Hiroyuki, 2003, Determination of macrolide antibiotics in meat and fish by liquid chromatography-electrospray mass spectrometry. *Analyt. Chimica Acta*, 492(1-2):187-197.

Michael H., Haiqing D., Robert M.C., Jane G.O. and P.H. Robert, 2007. Quantivative determination of tilmicosin in canine serum by high performance liquid chromatography tandem mass spectrometry. *J. Chromatogr. B*, 857(1):9-14.

Minh N.P., Lam T.B., Ciao N.T. and N.C. Quan, 2010, Tissue distribution and elimination of erythromycin in giant freshwater prawn (*Macrobrachium rosenbergii*) deleption. *Arican J. Food Sci.*, 4(9):578-584.

Minh N.P., Lam T.B., Ciao N.T. and N.C. Quan, 2011, Accumulation and clearance of orally administered erythromycin in adult Nile tilapia (*Oreocbromis niloticus*), International Food Research Journal 18,95-100.

Modric S., Webb A.I. and M. Davidson, 1999. Effect of Respiratory tract disease on pharmacokinetics of tilmicosin in rats. *Comparative Med.*, 49 (3):248-253.

Moran J.W., Turner J.M. and M.R. Coleman, 1997. Determination of tilmicosin in bovine and porcine sera by liquid chromatography. *J. AOAC Internat.*, 80(6):1183-1189.

Parker R.M., Patel R.K.P., Mclaren I.M. and P.G. Francis, 1994a. Residues of tilmicosin in milk of sheep after subcutaneous administration. Proceedings of the

European Association for Veterinary Pharmacology and Toxicology, 6th International Congress, Edinburgh, 227 pp.
Parker R.M., Patel R.K. P., Simmons H.A., 1994b. The pharmacokinetics of tilmicosin in sheep. Proceedings of the European Association for Veterinary Pharmacology and Toxicology, 6th International Congress, Edinburgh, 226-227 pp.
Plumb J.A., Sheifinger C.C., Shryock T.R. and T. Goldsby , 1995. Susceptibility of six bacterial pathogens of channel catfish to six antibiotics. *J. Aquat. Anim. Health*, 7:211-217.
Ramadan A., 1997. Pharmacokinetics of tilmicosin in serum and milk of goats. *Res. Vet. Sci.*, 62(1): 48-50.
Rérat M., Albini S., Jaquier V. and D. Hüssy, 2012. Bovine respiratory disease: Efficacy of different prophylactic treatments in veal calves and antimicrobial resistance of isolated Pasteurellaceae. *Prev. Vet. Med.*, 103(4):265-273.
Ritschel W.A., 1986. *Handbook of Basic Pharmacokinetic*. Drug Intelligence Publications, Hamilton. 544 pp.
Salvo A. D., Pellegrino R.M., Cagnardi P. and Rocca G. D., Pharmacokinetics and residue deleption of erythromycin in gilthead sea bream sparus aurata L. after oral administration. *J. Fish Dis.*, Doi:10.1111/jfd. 12170
Shen J.Z., Li C., Jiang H.Y., Zhang S.X., Guo P., Ding S.Y. and X.W. Li, 2005. Pharmacokinetics of tilmicosin after oral administration in swine. *Am. J. Vet. Res.,* 66 (6): 1071-1074.
Sun M., Li J., Gai CL., Chang ZQ., Li JT. and F.Z. Zhao, 2013. Pharmacokinetics of difloxacin in olive flounder Paralichthys olivaceus at two water temperature. *J. Vet. Pharmacol.Ther.*. doi: 10.1111/jvp.12062.
Tyrpenou AE., Kotzamanis Y.P. and M.N. Alexis, 2003. Flumequine depletion from muscle plus skin tissue of gilthead seabream (*Sparus aurata L*.) fed flumequine medicated feed in seawater at 18 and 24°C. *Aquaculture*, 220(1-4):633-642.
Watkins K.L., Shryock T.R., Dearth R.N. and Y.M. Saif, 1997. In vitro antimicrobial susceptibility of Clostridium perfringens from commercial turkey and broiler chicken origin. *Vet. Microbiol.*, 54(2):195-200.
Womble A., Giguère S., Murthy Y.V.S.N., Cox C. and E. Obare, 2006. Pulmonary dispotion of tilmicosin in foals and in vitro activity against Rhodococcus equi and other common equine bacterial pathogens. *J. Vet. Pharmacol Ther.*, 29(6):561-568.
Yamaoka, K. and T. Nakagawa, 1978. Application of Akaike's Information Criterion (AIC) in the evaluation of linear pharmacokinetic eaquations. *J. Phamacokinety Biop.*, 6(2):165-175.
Zhang Y., Jiang H., Jin X., Shen Z., Shen J., Fu C, and J. Guo, 2004. Residue Depletion of tilmicosin in chicken tissues. *J. Agricul. Food Chem.*, 52(9): 2602-2605.
Zhang J.Y.,Li J.Y., Miao X.L., Zhou X.Z., Li J.S., Li H.S., Dong P.C., Xu Z.Z. and X.X. Yan, 2004. Pharmacokinetics of tilmicosin in sheep. *Chinese J. Anim. Vet. Sci.*, 35(5):560-564.(in chinese).
Ziv, G., Shbm-Tov, M., Glickman, A., Winkler, M. and A. Saran, 1995. Tilmicosin antibacterial activity and pharmacokinetics in cows. *J. Vet. Pharmacol Ther*, 18(5): 340-345.