Pharmacokinetics of Florfenicol by Gavage Feeding or Medicated Feed in Rainbow trout (*Oncorhynchus mykiss*)

**Abstract**

The most cultivated freshwater species is rainbow trout in aquaculture worldwide. This study was aimed at pharmacokinetics of florfenicol administrated to rainbow trout (*Oncorhynchus mykiss*) by oral gavages (through a tube leading down the throat to the stomach) and medicated feed. 132 healthy rainbow trout weighing 140±10 g were randomly selected and after 2 weeks acclimation period, florfenicol were administrated as oral gavages and medicated feed at single dose 10 mg/kg body weight (B.W) to individual fish. Plasma samples were collected at 0, 0.5, 1, 3, 6, 9, 12, 24, 36, 48 and 72 h after feeding and analyzed by high performance liquid chromatography (HPLC) method. The data obtained from plasma concentrations of florfenicol after oral gavages and feed medicated routes were analyzed by SPSS version 16, Mann-Whitney U, ANOVA tests and (P < 0.05) was considered significant. The maximum concentration (C_{max}) was gained at 12 h for gavages route (4.68 µg/ml⁻¹) but at 9 h for medicated feed (6.1 µg/ml⁻¹). The maximum level concentration of florfenicol in medicated feed route was higher than oral gavages route (P < 0.05). It seems that feed can increase absorbance of florfenicol. Also, interestingly the C_{max} in medicated feed route rapidly reached and the decreasing process of drug showed less elimination in versus of time.

**Keywords:** Rainbow trout; Florfenicol; Pharmacokinetics; Gavages route; Medicated feed route

**Introduction**

The most cultivated freshwater species is rainbow trout in aquaculture worldwide. Fish production is annually increasing in Europe, Asia and American continents [1]. Germany, France, Italy, Iran and United States are countries which large production of fish has been registered [2]. In aquaculture, bacterial diseases are common with high density, so for prophylaxis or treatment of disease, the use of antibacterial agents is inevitable [3].

In the past, one of the main antibacterial agents in aquaculture was chloramphenicol which due to bone-marrow depression and irreversible aplastic anaemia in human, its use was limited until florfenicol, fluorinated analogue of thiamphenicol (Figure 1), synthesized and approved for veterinary use [4]. Approval of florfenicol had been done for use in fresh water-reared salmonids at 2007 by the U.S. Food and Drug Administration Centre for Veterinary Medicine. Florfenicol has great potential for treatment of bacterial infections of fish [5]. It is more active than thiamphenicol, chloramphenicol, and dangerous bacteria such as *Aeromonas salmonicida*, *Vibrio Salmonicida*, and *Edwardsiella ictaluri* are susceptible to florfenicol. Oral administration of florfenicol for treatment of bacterial infections of captive fish has been done under the trade names of Aquaflor® and Aquafen® in Canada and Europe, respectively [6].

Oral administration of florfenicol could be either as oral solution or medicated feed. Several studies have been done about pharmacokinetics of florfenicol in trout as oral and intramuscular administrations [7,8] but this study was aimed at pharmacokinetics of florfenicol following gavage and medicated feed administrations in rainbow trout.

**Materials and Methods**

**Chemicals**

Florfenicol standard was purchased from Sigma chemicals Co., USA and chloramphenicol standard was purchased from Merck Co, Germany. Stock standard solutions of florfenicol and chloramphenicol were prepared as 1 mg/ml⁻¹ and 0.5 mg/ml⁻¹ by dissolving each drug in methanol, respectively and stored at -20°C.

**Fish**

132 healthy rainbow trout (*Oncorhynchus mykiss*) weighing 140±10 g obtained from a trout breeding centre (2000 center, Tonekabon, Iran) were reared in fresh water. The fish were
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brought to cement tanks of Coldwater Fishes Research Centre which was disinfected 1 day prior to transmission. Water constant flow of 720 L/h\(^{-1}\) with oxygen content of (92±2) %, PH (7.2±0.1) and temperature of 14-15 °C in cement tanks were established. The fish were fed with pellet (%36-38 protein) in amount of 2% of the body weight TID for 2 weeks. After acclimation period (2 weeks), the fish were staved for 1 day before administration of drug.

Drug and drug administration

Florfenicol powder was donated by Behvazan Company (Tehran, Iran). Oral suspension of florfenicol (2.5mg/ml\(^{-1}\)) was prepared by dissolving florfenicol powder in propylene glycol. Medicated feed was prepared by blending the drug in feed (10mg/g 'feed') [7,8]. A single dose of 10 mg/kg 'body weight (B.W) florfenicol was given to individual fish.

Sampling

The samples were taken at 0, 0.5, 1, 3, 6, 9, 12, 24, 36, 48 and 72h after drug administration (oral gavages and medicated feed route). Blood samples were taken from caudal vein using a heparinized 2.5 ml syringe and plasma was isolated by centrifugation at 3000 rpm for 10 min. all samples were instantly frozen and stored at 20 °C until analysis.

Sample preparation

0.5ml plasma sample was added to 30µl chloramphenicol (2µg/ml\(^{-1}\)) for use as the internal standard. Each sample was whirl mixed for 2 min and then 3.5ml ethyl acetate was added and centrifuged at 3500 rpm for 1min to precipitate proteins. 2.5 ml supernatant was removed and evaporated to dryness under a gentle steam of nitrogen at 40 °C. The residue was dissolved in 0.5ml of mobile phase solution (water - acetonitrile, 75:25, v/v) and after centrifugation, filtered through a syringe filter (0.45µm), and 100µl were injected on the HPLC column.

Chromatographic condition

The analyses were performed by HPLC system (waters 2695, U.K), consisted of a reaction pump, Intelligent pump and waters 486 detector at 234 nm. The separation was performed at 40 °C on a 200 mm × 4.6 mm I.D ODS-A column packed with 5 µm, 120-A and 100µl were injected on the HPLC column.

The maximum level concentration of florfenicol in medicated feed route was higher than gavage route .It seems that feed can increase absorbance of florfenicol.

Also, interestingly the maximum level concentration in medicated feed route rapidly reached. Meinertz et al. [10] have reported florfenicol concentration in skin-on fillets in the recirculating aquaculture system (RAS) 11.50µg/ml\(^{-1}\) at 13 °C. T\(_{\text{max}}\) of 12h in plasma oral gavages route was in agreement with the results of Feng et al. [6] in tilapia and Martinsen et al. [11] in Atlantic salmon after oral dosing at 10mg/kg\(^{-1}\) florfenicol. In this study, the decreasing process of drug in medicated feed route showed less elimination in versus of time. Martinsen et al. [11] have reported rapid absorption and less elimination in medicated feed. Feed medicated route is a safe method with high efficiency.
which is confirmed by Straus et al. [12] and is in agreement with our study. Cao et al. [13] have reported $C_{\text{max}}$ 10.8µg/ml⁻¹ after 8 h in top mouth culter (Culter alburnus) which was higher than our results. In conclusion, the present study showed that florfenicol as medicated feed route was better than gavage route both higher and rapid concentration in rainbow trout.

Table 2: Pharmacokinetics parameters for florfenicol after oral gavages and medicated feed routes.

| Dose | $C_{\text{max}}$ (µg/ml⁻¹) | $T_{\text{max}}$ (h) |
|------|----------------|-----------------|
| Oral Administration | 10 | 4.68 | 12 |
| Gavage route | 10 | 6.1 | 9 |

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Conflict of Interest

None.

References

1. Biancotto G, Contiero L, Benetti C, Calligaris M, Tibaldi E, et al. (2009) Depletion of chloramphenicol in trout after a hypothetic therapeutic treatment. Analytica Chimica Acta 637(1-2): 173-177.
2. Food and Agriculture Organization in the United States fisheries and aquaculture department. 2011. Cultured Aquatic Species Information program Oncorhynchus mykiss (walbaum, 1792).
3. Lu XW, Dang Z, Yang C (2009) Preliminary investigation of chloramphenicol in fish, water and sediment from freshwater aquaculture pond. Int J Environ Sci Technol 6(4): 597-604.
4. Carty D, Bowker JD, Bowman MP, Erdahl DA (2007) Calculate amount of aquaflo (florfenicol, 50%) to add to fish feed. Drug Research Information Bulletin of the U.S Fish and Wildlife Service. Aquatic Animal Drug Approval Partnership.
5. USFDA CVM (U.S Food and Drug Administration, center for veterinary medicine) (2006) Guidance for industry. General principles for evaluating the safety of compounds used in food - producing animals. U.S Department of Health and Human Services.
6. Feng JB, Li XP, Li LD (2008) Tissue distribution and elimination of florfenicol in tilapia (Oreochromis niloticus × O. caureus) after a single oral administration in freshwater and seawater at 28°C. Aquaculture 276(1-4): 29-35.
7. Park Bk, Lim JH, Kim MS, Yun HI (2006) Pharmacokinetics of florfenicol and its metabolite, florfenicol amine, in the Korean catfish (Silurus asotus). J Vet Pharmacol Ther 29(1): 37-40.
8. Horsberg TE, Martinson B, Varma KJ (1994) The disposition of 14C-florfenicol in Atlantic salmon (Salmo salar), Aquaculture 122(2-3): 97-106.
9. Yanong RPE, Curtis RW, Simmons R, Bhattaram VA, Gopalakrishnan M, et al. (2005) Pharmacokinetic studies of florfenicol in koi carp and three spot gourami (Trichogaster trichopterus) after oral and intramuscular treatment. J Aquatic Animal Health 17(2): 129-137.
10. Meinertz JR, Schreier TM, Bernardy JA (2008) Evaluation of a method for determining concentrations of isoeugenol, an AQUI-S residue, in fillet tissue from freshwater fish species. J AOAC Int 91(4): 884-891.
11. Martinson B, Horsberg TE, Varma KJ, Sims RA (1993) Single dose pharmacokinetic study of florfenicol in Atlantic salmon (Salmo salar) in seawater at 11°C, Aquaculture 112(1): 1-11.
12. Straus LD, Bowker JD, Bowman MP, Carty D, Mitchell AG, et al. (2012) Safety of aquaflor-Medicated Feed to Sunshine Bass, North American Journal of Aquaculture 74(1): 1-7.
13. Cao XJ, Wang WM, Song F (2011) Anatomical and Histological Characteristics of the intestine of the Top mouth culter (Culter alburnus). Anatomia Histologia Embryologia 40(4): 292-298.

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