

Florfenicol Medicated Feed (Aquaflor®)Clinical Field Trials - INAD 10-697

Year 2010 Annual Summary Report on the Use of Florfenicol Medicated Feed in Field Efficacy Trials

Prepared by:

Bonnie Johnson, Biologist
U.S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, Montana

Summary

Florfenicol-medicated feed (Aquaflor®)(FMF) has been used effectively in the U.S. under compassionate INAD Exemption #10-697 to control mortality in a variety of fish caused by common fish bacterial pathogens. In calendar year 2010 the efficacy of FMF was evaluated in 52 disease trials involving approximately 7.1 million fish to control mortality in a variety of test fish caused by a variety of infectious fish pathogens. Trials were conducted at a total of 20 fish culture facilities, including two U.S. Fish and Wildlife Service National Fish Hatcheries (NFH), two state hatcheries, and 16 private hatcheries. Use of FMF under Protocol #10-697 allowed the investigator to administer FMF at either a dosage of 10 mg/Kg fish/day for 10 days or 15 mg/Kg fish/day for 10 days. Overall results indicated that treatment appeared effective in approximately 88% of the trials, ineffective in 6% of the trials, and was characterized as inconclusive in 6% of the trials.

Introduction

The current labels for FMF use in aquaculture limits use to: 1) the control of furunculosis in salmonids caused by *Aeromonas salmonicida*; 2) control of coldwater disease in salmonids caused by *Flavobacterium psychrophilum*; 3) control of enteric septicemia in catfish caused by *Edwardsiella ictaluri*; and 4) control of columnaris in catfish caused by *Flavobacterium columnare*. These label restrictions limit the overall utility of approved FMF use in aquaculture.

Bacterial diseases are a major problem in aquaculture and account for significant losses of fish (Clarke and Scott 1989; Frerichs and Roberts 1989; Bjorndal 1990). Although the importance of environmental conditions (McCarthy and Roberts 1980; Haastein 1988; Munro and Roberts 1989) and the value of effective vaccines, where available (Ellis 1989), are acknowledged, antimicrobial therapy presently has an important role to play in aquaculture (Klontz 1987; Alderman 1988). Florfenicol is a potent, broad-spectrum, antimicrobial agent with bacteriostatic properties (Horsberg et al. 1996). It is a fluorinated analogue of thiamphenicol and is also similar in structure to chloramphenicol, both of which have been used as broad-spectrum, veterinary antibiotics (Nagata and Oka 1996).

Florfenicol has great potential for treatment of infectious diseases, and because of its high potency and safety to humans, it could become an important drug in veterinary medicine, especially with respect to animals used by humans for food

(Powers et al. 1990). Additionally, because florfenicol is not currently used in human medicine, it has become a strong candidate for use in aquaculture, and there is considerable interest to obtain U.S. Food and Drug Administration (FDA) approval for its use in fish culture.

The proposed treatment strategy (i.e., dosage and duration) for the use of FMF in fish is designed to meet the needs of individual fish species, individual fish lots, and a variety of environmental conditions. In all cases, treatment goals are to (1) minimize the negative effects of disease on fish health, quality, and survival, and (2) help meet fishery management objectives. Because many factors can affect the success or failure of florfenicol-medicated feed therapy, supplemental efficacy data from compassionate Investigational New Animal Drug (INAD) use, as well as efficacy data from controlled, replicated studies that are scientifically valid and statistically defensible (i.e., pivotal), are needed to gain approval of FMF use in aquaculture.

Purpose of Report

The purpose of this report is to summarize the results of supplemental FMF field efficacy studies conducted in calendar year 2010 (CY10) . Furthermore, it is expected that these data will be used to enhance the FMF database for the purpose of expanding an appropriate label claim for the use of this new animal drug.

Facilities, Materials, and Treatment Procedures

1. Participating Facilities

A total of 52 effectiveness trials were conducted at 20 fish culture facilities, including two U.S. Fish and Wildlife Service National Fish Hatchery (NFH), two state hatcheries, and 16 private hatcheries. Trials were conducted to control mortality in a variety of fish caused by a variety of fish pathogens. Water temperature during treatment trials ranged from 50.0 - 90.0 °F, with a mean treatment temperature of 70.0°F.

2. FMF used in trials

The Aquaflor[®] used in CY10 trials contained 500 g of florfenicol per kg of premix. Florfenicol is a pure compound with no inactive ingredients. All florfenicol used was supplied as Aquaflor[®] by Schering-Plough Animal Health, 1095 Morris Avenue, Union, NJ. Florfenicol medicated feed was prepared either by top-coating florfenicol onto commercial fish feed at the testing site by the Investigator, Monitor, or their designee, or prepared by commercial fish feed manufacturers.

3. Drug dosages and duration

As described in the Study Protocol for INAD #10-697, Investigators were allowed to use FMF at either a dosage of 1) 10 mg/Kg fish/day for 10 days (approximately 33% of trials were conducted using this treatment regimen) or 2) 15 mg/Kg

fish/day for 10 days (approximately 60% of trials were conducted using this treatment regimen).

Study Protocol Deviation: Treatment regimen administered in the remaining trials (approximately 7% of the trials) deviated from the protocol. Protocol deviations occurred at three facilities where the fish were either treated at 1) 7.8 mg/kg fish/day for 10 days - lower dosage used due to a calculation error by the investigator; 2) 10 mg/kg fish/day for 5 days - the investigator ended the treatment early due to mortality returning to zero; 3) 10 mg/kg fish/day for 10 non consecutive days - due to a harmful algae bloom in the area 2 - 3 days after treatment started the feeding was stopped until the waters cleared up; or 4) 30 mg/kg fish/day for 10 days - higher dosage used due to a calculation error by the investigator. The investigators and monitors were contacted in each of these deviations and reminded to follow the study protocol. In the cases where the fish were treated at 7.8 and 30 mg/kg fish/day the AADAP Office had sent the correct calculations along with the Aquaflor[®] pre mix; however, the individual responsible for mixing up the feed did not follow these calculations. Measures are now in place to ensure calculation errors will not occur for future studies.

Fish Species and Fish Diseases Involved in CY10 Trials

1. Species of fish treated

Twelve fish species, including two species of salmonids, five non-salmonid species, and five marine non-salmonid species were treated with FMF during

CY10. Treated fish ranged in length from 1.0 - 32.0 in. and the mean length of all treated fish was 7.6 in. Fish species treated included:

Salmonids:

Atlantic salmon *Salmo salar*

cutthroat trout *Oncorhynchus clarki*

Non-salmonids:

channel catfish *Ictalurus punctatus*

koi *Cyprinus carpio*

largemouth bass *Micropterus salmoides*

muskellunge *Esox masquinongy*

Tilapia *Oreochromis niloticus*

Marine non-salmonids:

barramundi *Lates calcarifer*

European sea bass *Dicentrarchus labrax*

gilthead seabream *Sparus aurata*

kona kampachi *Seriola rivoliana*

summer flounder *Paralichthys dentatus*

2. Diseases treated

Test fish were treated with FMF to control mortality caused by the following diseases during CY10: *Aeromonas hydrophila*; *Carnobacterium piscicola*; *columnaris*; *Edwardsiella tarda*; flavobacteriosis; furunculosis; motile aeromonad septicemia; piscirickettsia; rickettsia like organism; streptococcus; *Streptococcus iniae*; *Vibrio alginolyticus*; *Vibrio harveyi*; *Vibrio ichthyenteri*; vibriosis; and yellow mouth.

Data Collected

1. Pathology reports

Pathology reports were submitted with 22 trials conducted during CY10. Fish health pathology reports included: 1) a description of how the identity of disease agent(s) was verified; 2) disease identification records that confirm the presence of the disease agent; and 3) the name and title of the individual performing the diagnosis. Additionally, pathology reports often provide documentation that there were no secondary infections or infestations caused by unrelated disease agents in the population of test fish. Pathology reports provide critical information if such submissions are to be used in support of an initial approval, or to expand/extend an existing approved label.

2. Mortality data

As stated in the Study Protocol, mortality data were to be collected 10 days prior to treatment, during the treatment period, and for at least 21 days post-treatment.

Investigators were strongly encouraged to collect mortality data on a daily basis. However, daily collection of pre-treatment mortality data was not always possible due to fish being moved (i.e., split into additional rearing units, or combined with fish from another rearing unit) from rearing unit to rearing unit.

Discussion of Study Results:

1. General observations on the efficacy of FMF for the control of bacterial

diseases in salmonid and non-salmonid fish (Note: Table 1 provides a list of all efficacious trials; Table 2 provides a list of all ineffective trials; Table 3 provides a list of all inconclusive trials; Table 4 provides general CY10 summary data; and Table 5 provides a summary of all trials conducted during CY10 under INAD #10-697.

A. Salmonid species - efficacy at 10 - 15 mg/Kg fish/d for 10 days under INAD #10-697

Atlantic salmon and cutthroat trout were treated with 10 - 15 mg florfenicol/Kg fish/d for 10 days in 17 trials to control mortality caused by *Carnobacterium piscicola*, piscirickettsia, rickettsia like organism, or yellow mouth (Tables 1 - 3). FMF treatments appeared effective in 15 trials; ineffective in one trial; and inconclusive in one trial.

B. Non-salmonid species - efficacy at 10 - 15 mg/Kg fish/d for 5 - 10 days under INAD #10-697

Channel catfish, koi, largemouth bass, muskellunge, and Tilapia were treated with 10 - 15 mg florfenicol/Kg fish/d for 5 - 10 days in 21 trials to control mortality caused by *Aeromonas hydrophila*; *columnaris*; *Edwardsiella tarda*; flavobacteriosis; furunculosis; motile aeromonad septicemia; or *Streptococcus iniae*, (Tables 1 & 3). FMF treatments appeared effective in 19 trials and were characterized as inconclusive in two trials.

C. Marine non-salmonid species - efficacy at 7.8 - 30 mg/Kg fish/d for 10 days under INAD #10-697

Barramundi, European sea bass, gilthead seabream, Kona kampachi, and summer flounder were treated with 7.8 - 30 mg florfenicol/Kg fish/d for 10 days in 14 trials to control mortality caused by *Edwardsiella tarda*, streptococcus, *Streptococcus iniae*, *Vibrio alginolyticus*, *Vibrio harveyi*, *Vibrio ichthyenteri*, or vibriosis (Tables 1 - 2). FMF treatments appeared effective 12 trials and were ineffective in two trials.

2. Observed Toxicity

No toxicity or adverse effects relating to FMF treatment were reported in 51 trials. In the trial where the summer flounder were treated at 30 mg/kg fish/day, initial toxicity seemed evident in the smallest fish on the 3rd day of treatment.

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded in all trials.

Current Study Protocol for FLOR (Aquaflor®) INAD #10-697

Please see the attached current study protocol for FLOR (Aquaflor®) INAD #10-697. Please note no changes have occurred to this study protocol.

Facility Sign-up List

Please see “Table 6. Facilities and Names of Investigators” for facilities that signed-up to participate in the FLOR (Aquaflor®) INAD #10-697 during CY10. Facilities not listed in Appendix III-a of the current FLOR (Aquaflor®) INAD #10-697 during CY10 study protocol have been highlighted. Please note all of these facilities are in compliance with their reporting requirements to the NPDES authority.

Correspondence sent to FLOR (Aquaflor®) Participants

Please see the attached correspondence that was sent to all FLOR (Aquaflor®) participants after the AADAP Office received their sign-up form for CY10.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY10 was 7,084,774. The total number of treated fish to count against treatment use authorization dated July 31, 2009 is 10,876,767.

Summary of Study Results

Florfenicol medicated feed (Aquaflor[®]) was administered to test fish in 52 separate trials at dosages ranging between 7.8 - 30 mg/Kg fish/d for 5 - 10 d. Twelve different fish species were treated with FMF, and trials involved approximately 7.1 million fish. Treated fish ranged in size from 1.0 - 32.0 in. Water temperature during treatment ranged from 50.0 - 90.0°F, with a mean treatment temperature of 70.0°F. Overall results showed that in approximately 88% of the trials, FMF treatments appeared effective, 6% of the trials were ineffective, and 6% of the trials were characterized as inconclusive. Although data from these trials will be considered ancillary, trial results should provide useful corroborative data to support a new/expanded label claims for FMF. It is anticipated that additional ancillary efficacy data will continue to be collected under INAD #10-697. In future trials conducted under INAD #10-697, efforts will continue to be directed towards the generation of high quality data.

References

- Alderman, D. J. 1988. Fisheries chemotherapy: a review. In: Recent Advances in Aquaculture, Vol. 3. Croom Helm, London.
- Bjorndal, T. 1990. The economics of salmon aquaculture. Blackwell Scientific Publications, Oxford.
- Clarke, R., and D. Scott. 1989. An overview of world salmon production and recent technology developments. Bulletin of the Aquaculture Association of Canada 4:31-48.
- Ellis, A. E. 1989. Use of vaccines in controlling fish diseases. Developmental and Comparative Immunology 13:399-407.

- Frerichs, G. N., and R. J. Roberts. 1989. The bacteriology of teleosts. In Fish Pathology, 2nd edition. Balliere Tindall, London.
- Haastein, T. 1988. Disease control through management practices. In: Proceedings of the Aquaculture International Congress, P. 39. Aquaculture International Congress, Vancouver, B.C.
- Horsberg, T. E., K. A. Hoff, and R. Nordmo. 1996. Pharmacokinetics of florfenicol and its metabolite florfenicol amine in Atlantic salmon. Journal of Aquatic Animal Health 8:292-301.
- Klontz, G. W. 1987. Control of systemic bacterial diseases in salmonids. Salmonid. 11:5-13.
- McCarthy, D. H. and R. J. Roberts. 1980. Furunculosis of fish - the present state of our knowledge. In: Advances in Aquatic Microbiology. Academic Press, London.
- Munro, A. L. S. and R. J. Roberts. 1989. The aquatic environment. In: Fish Pathology, 2nd edition. Balliere Tindall, London.
- Nagata, T. and H. Oka. 1996. Detection of residual chloramphenicol, florfenicol, and thiamphenicol in yellowtail fish muscles by capillary gas chromatography-mass spectrometry. Journal of Agriculture Food Chemistry 44:1280-1284.
- Powers, T. E., K. J. Varma, and J. D. Powers. 1990. In F. Simon (editor). Abstracts of the 4th Congress of European Associations of Veterinary Pharmacology and Toxicology, Budapest, Aug 28-Sep 2, 1988. Vol. 1, University of Veterinary Science, Budapest, 1990.

Table 1. CY10 Florfenicol Medicated Feed (Aquaflor®) Efficacy Results - Effective Trials - cont

Number of Studies	Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Temp. (°F)
1	Clam Bay Orchard Rocks Fort Ward	ATS	32.00	122,945	Piscirickettsia	10	10	55.0
1	Hope Island	ATS	10.60	753,620	Yellow Mouth	15	10	52.1
13	Port Angeles	ATS	4.00	2,793,939	Yellow Mouth	15	10	50.0
2	Australis - Production Facility	BMU	5.9 - 6.7	222,974	Streptococcus iniae	15	10	80.0 - 81.4
4	Brady Catfish	CCF	10.0 - 12.0	750,000	Aeromonas hydrophila	10	10	90.0
1	Inks Dam NFH	CCF	2.50	150,876	Motile Aeromonad Septicemia	10	10	84.2
3	Steve Miller Farms	CCF	12.00	330,000	Aeromonas hydrophila	10	5 - 10	90.0
2	Wilmer Penner Catfish Farms	CCF	10.0 - 12.0	150,000	Aeromonas hydrophila	10	10	78.0 - 86.0
2	Local Ocean	ESB	3.0 - 4.0	109,405	Vibrio harveyi	15	10	70.0 - 73.0
3	Local Ocean	GSE	4.0 - 8.0	173,256	Edwardsiella Tarda	7.8 - 15	10	68.0 - 71.6
1	Local Ocean	GSE	7.00	28,062	Vibrio ichthyenteri	15	10	70.0
1	Lahser Research	KOI	24.00	118	Furunculosis	15	10	72.0
1	Keahole Point - Offshore Mariculture Site	KON	5.90	120,000	Vibrio alginolyticus	10	10	78.4
1	Kona Blue Water Farms	KON	6.00	88,000	Streptococcus	10	10	72.0
1	Kona Blue Water Farms	KON	8.00	100,000	Vibrosis	10	10	72.0
1	Cain Fish Farm	LMB	7.00	15,000	Edwardsiella Tarda	15	10	83.0
1	Manning SFH	LMB	2.00	18,000	Columnaris	15	10	69.0
1	Robert Mayer Facility	LMB	8.00	79,995	Furunculosis	15	10	83.0
1	Seven Springs Fish Farm	LMB	12.00	40,000	Columnaris	10	10	85.0
4	MinAqua Fisheries	NTI	1.0 - 6.0	277,548	Streptococcus iniae	15	10	80.0 - 83.0
1	Local Ocean	SFL	5.00	11,180	Vibrio ichthyenteri	30	10	68.0

Table 2. Summary of CY10 Florfenicol Medicated Feed Efficacy (Aquaflor®) Results - Ineffective Trials

Number of Studies	Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Temp. (°F)
1	Lahontan NFH	CUT	13.50	791	Carnobacterium piscicola	15	10	54.0
1	Local Ocean	ESB	4.00	142,016	Edwardsiella Tarda	15	10	72.0
1	Keahole Point - Offshore Mariculture Site	KON	12.20	120,000	Vibriosis	10	10	78.4

Table 3. Summary of CY10 Florfenicol Medicated Feed Efficacy Results - Inconclusive Trials

Number of Studies	Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Temp. (°F)
1	Cypress Island	ATS	30.00	208,758	Rickettsia Like Organism	10	10	52.5
1	Wolf Lake SFH	MUE	4.00	240,000	Flavobacteriosis	10	10	72.0
1	MinAqua Fisheries	NTI	1.00	38,291	Streptococcus iniae	15	10	83.0

Table 4. Summary Data Regarding CY10 Florfenicol Medicated Feed (Aquaflor®) Efficacy Trials

Total Fish Treated:		<u>7,084,774</u>
Number of fish treated in effective trials		6,334,918
Number of fish treated in ineffective trials		262,807
Number of fish treated in inconclusive trials		487,049
Total number of trials:		52
Number of effective trials:		46
Number of ineffective trials:		3
Number of inconclusive trials:		3
Treatment Regimes Used:		
7.8 mg/Kg fish/day for 10 days		1 trial
10 mg/Kg fish/day for 5 - 10 days		19 trials
15 mg/Kg fish/day for 10 days		31 trials
30 mg/Kg fish/day for 10 days		1 trial
Treatment Water Temperature (°F):		
Temperature Range	50.0 - 90.0	
Mean Temperature	70.0	
Size of Treated Fish (in.):		
Size Range	1.0 - 32.0	
Species Treated:		
<u>Salmonids:</u>		
Atlantic salmon <i>Salmo salar</i>		
cutthroat trout <i>Oncorhynchus clarki</i>		
<u>Non-salmonids:</u>		
channel catfish <i>Ictalurus punctatus</i>		
koi <i>Cyprinus carpio</i>		
largemouth bass <i>Micropterus salmoides</i>		
muskellunge <i>Esox masquinongy</i>		
Tilapia <i>Oreochromis niloticus</i>		
<u>Marine non-salmonids:</u>		
barramundi <i>Lates calcarifer</i>		
European sea bass <i>Dicentrarchus labrax</i>		
gilthead seabream <i>Sparus aurata</i>		
kona kampachi <i>Seriola rivoliana</i>		
summer flounder <i>Paralichthys dentatus</i>		