

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

Year 2008 Annual Summary Report on the Use of Florfenicol Medicated Feed in Field Efficacy Trials

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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 2008 the efficacy of FMF was evaluated in 66 disease trials involving approximately 8.5 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 one U.S. Fish and Wildlife Service National Fish Hatchery (NFH), seven state hatcheries, and 12 private hatcheries. Use of FMF under Protocol #10-697 allowed the investigator to administer FMF at a dosage of 10 mg/Kg fish/day for 10 days. Overall results indicated that treatment appeared effective in approximately 79% of the trials, ineffective in 6% of the trials, and was characterized as inconclusive in 15% 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 2008 (CY08) . 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 66 effectiveness trials were conducted at 20 fish culture facilities, including one U.S. Fish and Wildlife Service National Fish Hatchery (NFH), seven state hatcheries, and 12 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 44.0 - 85.0 °F, with a mean treatment temperature of 72.8°F.

2. FMF used in trials

The Aquaflor[®] used in CY08 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 10 mg of active drug/kg of fish/d for 10 d (approximately 86% of trials were conducted using these treatment regimens).

Study Protocol Deviation: Treatment regimen administered in the remaining trials (approximately 14% of the trials) deviated from the protocol. Note: both deviations were reported to CVM in the florfenicol quarterly report dated July 1, 2008.

- 1) The first deviation occurred at a private hatchery and involved largemouth bass. The fish were treated 17 days (spread over a 45 day period) due to the fish not eating the allotted amount of feed because of cold water temperatures. This caused the dosage to calculate out to 5.6 mg/Kg fish/day. The fish did not enter the human food chain for 45 days after the last treatment day. The Investigator was contacted and future studies will follow the study protocol.

- 2) The second deviation occurred at a state facility where the treatment dose ranged between 20 mg/Kg fish/day to 50 mg/Kg fish/day for 10 consecutive days. The treated fish were a variety of ornamental fish and will never enter the human food chain. Please note that a letter requesting an amended authorization for this deviation had been submitted to CVM on 6/27/08. However, CVM advised a separate protocol would be needed for these fish. The investigator was contacted and it was determined the studies would not continue due to the principle investigator leaving the project.

Fish Species and Fish Diseases Involved in CY08 Trials

1. Species of fish treated

Sixteen fish species, including four species of salmonids, four non-salmonid species, two marine species, and six tropical (aquarium) species were treated with FMF during CY08. Treated fish ranged in length from 0.82 - 22.0 in. and the mean length of all treated fish was 5.5 in. Fish species treated included:

Salmonids:

Atlantic salmon *Salmo salar*

brown trout *S. trutta*

brook trout *Salvelinus fontinalis*

rainbow trout *Oncorhynchus mykiss*

Non-salmonids:

channel catfish *Ictalurus punctatus*

hybrid striped bass *Morone americana* x *M. saxatilis*

largemouth bass *Micropterus salmoides*

Tilapia *Oreochromis niloticus*

Marine non-salmonids:

kona kampachi *Seriola rivoliana*

southern flounder *Paralichthys lethostigma*

Tropical (Aquarium) Species:

albino cory catfish *Corydoras aeneus*

angelfish *Pterophyllum scalare*

Cichlidae spp.

longfin danios *Brachydanio rerio*

neon tetras *Paracheirodon innesi*

swordtail *Xiphophorus helleri*

2. Diseases treated

The disease treated most frequently was characterized as *Streptococcus iniae* (44% of the trials). Other diagnosed diseases included: *Aeromonas hydrophila*, *Aeromonas* spp., *Carnobacterium* spp., *columnaris*, *Edwardsiella tarda*, general systemic bacterial infection, motile *Aeromonad*, vibriosis, and yellow mouth (*Tenacibaculum maritimum*).

Data Collected

1. Pathology reports

Pathology reports were submitted with 11 trials conducted during CY08. 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 summary of all efficacious trials; Table 2 provides a summary of all ineffective trials; Table 3 provides a summary of all inconclusive trials; Table 4 provides general CY08 summary data; and Table 5 provides a summary of all trials conducted during CY08 under INAD #10-697.

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

Atlantic salmon, brook, brown, and rainbow trout, were treated with 10 mg florfenicol/Kg fish/d for 10 days in seven trials to control mortality caused by *Aeromonas hydrophila*, *Aeromonas* spp., *Carnobacterium* spp., columnaris, Motile Aeromonad, or yellow mouth (Tables 1 - 3). FMF treatments appeared effective in four trials; ineffective in two trials; and were characterized as inconclusive in one trial.

B. Non-salmonid species - efficacy at 5.6 - 10 mg/Kg fish/d for 10 - 17 days under INAD #10-697

Channel catfish, hybrid striped bass, largemouth bass, and tilapia were treated with 5.7 - 10 mg florfenicol/Kg fish/d for 10 - 17 days in 39 trials to control mortality caused by *Aeromonas hydrophila*, *Aeromonas* spp., columnaris, *Edwardsiella tarda*, motile aeromonad septicemia, or *Streptococcus iniae* (Tables 1 & 3). FMF treatments appeared effective in 35 trials and were characterized as inconclusive in four trials.

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

Kona kampachi and southern flounder were treated with 10 mg florfenicol/Kg fish/d for 10 days in 10 trials to control mortality caused by vibriosis or *Streptococcus iniae* (Table 1). FMF treatments appeared effective in all trials.

D. Tropical (Aquarium) species - efficacy at 10 - 50 mg/Kg fish/d for 10 days under INAD #10-697

Albino cory catfish, angelfish, Cichlidae spp., longfin danios, neon tetras, and Swordtail were treated with 10 - 50 mg florfenicol/Kg fish/d for 10 days in 10 trials to control mortality caused by *Aeromonas* spp., *columnaris*, general systemic bacterial infection, or vibriosis (Tables 1 - 3). FMF treatment appeared effective in three trials, ineffective in two trials, and were characterized as inconclusive in five trials.

2. Observed Toxicity

No toxicity or adverse effects relating to FMF treatment were reported.

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 CY08.

The following facility had Aquaflor[®] premix on-hand during CY08 but never used the drug:

1. Eagle Fish Health Lab

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 calendar year 2008.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY08 was 8,539,312. The total number of treated fish to count against the treatment use authorization dated September 17, 2007 is 9,196,971.

Summary of Study Results

Florfenicol medicated feed (Aquaflor[®]) was administered to test fish in 66 separate trials at dosages ranging between 5.6 - 50 mg/Kg fish/d for 10 - 17 d. Sixteen different fish species were treated with FMF, and trials involved approximately 8.5

million fish. Treated fish ranged in size from 0.8 - 22 in. Water temperature during treatment ranged from 44 - 85 °F, with a mean treatment temperature of 72.8 °F. Overall results showed that in approximately 79% of the trials, FMF treatments appeared effective, 6% of the trials were ineffective, and 15% 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. Summary of CY08 Florfenicol Medicated Feed (Aquaflor®) Efficacy Results - Effective Trials

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease	Number of Treatment Days	Dose (mg/kg)	Temp. (°F)
Port Angeles	1	ATS	6.00	1,088,273	Yellow Mouth	10	10	49.0
Oden SFH	1	BNT	22.00	1,270	Carnobacterium spp.	10	10	45.7
Inks Dam NFH	4	CCF	5.0 - 7.5	44,859	Motile Aeromonad Septicemia	10	10	68.9 - 75.3
Kona Blue Water Farms	1	KON	7.90	57,215	Vibriosis	10	10	78.8
	5	KON	7.1 - 9.8	250,840	<i>Streptococcus iniae</i>	10	10	78.8
JM Malone Facility	1	LMB	2.50	1,200,000	<i>Edwardsiella tarda</i>	10	10	60.0
	1	LMB	1.50	2,023,200	Aeromonas spp.	10	10	65.0
	1	LMB	2.00	200,000	Columnaris	10	10	80.0
Richloam SFH	3	LMB	1.9 - 2.6	61,780	Columnaris	10	10	72.9 - 75.0
Seven Springs Fish Farm	3	LMB	1.5 - 10.0	808,000	Columnaris	10	10	50.0 - 83.0
Bellingham SFH	1	RBT	3.40	25,137	Columnaris	10	10	68.0
Thompson SFH	1	RBT	3.30	71,800	Aeromonas spp.	10	10	45.0
Fish Barn	4	STF	3.0 - 10.0	39,000	Vibriosis	10	10	73.0
Kent SeaTech Corp.	7	SXW	2.0 - 10.6	1,753,395	<i>Streptococcus iniae</i>	10	10	79.0
Brock Farms	2	TIA	10.0 - 10.7	30,271	<i>Streptococcus iniae</i>	10	10	74.0 - 76.0
Circle G Farm	2	NTI	10.00	118,646	<i>Streptococcus iniae</i>	10	10	76.0 - 77.1

Table 1. Summary of CY08 Florfenicol Medicated Feed (Aquaflor®) Efficacy Results - Effective Trials - cont.

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease	Number of Treatment Days	Dose (mg/kg)	Temp. (°F)
Koehn Tilapia Production	1	NTI	5.00	80,000	<i>Aeromonas Hydrophila</i>	10	10	59.0
MinAqua Fisheries	6	NTI	1.5 - 6.0	337,261	<i>Streptococcus iniae</i>	10	10	80.0 - 83.0
Pridgen	2	NTI	5.0 - 5.2	45,755	<i>Streptococcus iniae</i>	10	10	77.0
Triple M Aquaculture	2	TIA	10.00	88,121	<i>Streptococcus iniae</i>	10	10	75.0 - 76.0
Tropical Aquaculture Lab	1	NTR	0.82	300	Vibriosis	10	20	78.0
Tropical Aquaculture Lab	1	ACC	1.85	69	Columnaris	10	50	83.0
	1	NTR	0.87	600	Vibriosis	10	50	78.0

Table 2. Summary of CY08 Florfenicol Medicated Feed Efficacy (Aquaflor®) Results - Ineffective Trials

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease	Number of Treatment Days	Dose (mg/kg)	Temp. (°F)
Watersmeet Trout Hatchery	1	BKT	2.00	10,000	<i>Aeromonas Hydrophila</i>	10	10	44.0
Tropical Aquaculture Lab	2	NTR	0.8 - 0.9	743	Vibriosis	10	10	77.0 - 78.0
Watersmeet Trout Hatchery	1	RBT	2.00	10,000	<i>Aeromonas Hydrophila</i>	10	10	44.0

Table 3. Summary of CY08 Florfenicol Medicated Feed Efficacy Results - Inconclusive Trials

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease	Number of Treatment Days	Dose (mg/kg)	Temp. (°F)
Seven Springs Fish Farm	1	LMB	12.00	27,000	<i>Edwardsiella tarda</i>	17	5.6	45.0
Marquette SFH	1	BKT	3.90	59,524	Motile Aeromonad	10	10	46.0
Inks Dam NFH	1	CCF	7.06	10,177	Motile Aeromonad Septicemia	10	10	73.2
Richloam SFH	1	LMB	4.90	15,600	Columnaris	10	10	78.0
Koehn Tilapia Production	1	NTI	7.00	80,000	<i>Edwardsiella tarda</i>	10	10	64.0
Tropical Aquaculture Lab	1	ANG	2.70	36	Aeromonas spp.	10	50	78.0
	1	CIC	1.30	150	General Systemic Bacterial Infection	10	50	77.0
	1	LFD	1.80	110	Vibriosis	10	50	83.0
	1	SWT	2.00	69	Columnaris	10	50	82.0
	1	SWT	2.20	111	Aeromonas spp.	10	50	85.0

Table 4. Summary Data Regarding CY08 Florfenicol Medicated Feed (Aquaflor®) Efficacy Trials

Total Fish Treated:	<u>8,539,312</u>
Number of fish treated in effective trials	8,325,792
Number of fish treated in ineffective trials	20,743
Number of fish treated in inconclusive trials	192,777

Total number of trials:	66
Number of effective trials:	52
Number of ineffective trials:	4
Number of inconclusive trials:	10

Treatment Regimes Used:

10 mg/Kg fish/day for 10 days	57 trials
5.6 mg/Kg fish/day for 17 days	1 trial
20 mg/Kg fish/day for 10 days	1 trial
50 mg/Kg fish/day for 10 days	7 trials

Treatment Water Temperature (°F):

Temperature Range	44.0 - 85.0
Mean Temperature	72.8

Size of Treated Fish (in.):

Size Range	0.82 - 22.0
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Species Treated:

Salmonids:

Atlantic salmon *Salmo salar*
brown trout *S. trutta*
brook trout *Salvelinus fontinalis*
rainbow trout *Oncorhynchus mykiss*

Non-salmonids:

channel catfish *Ictalurus punctatus*
hybrid striped bass *Morone americana* x *M. saxatilis*
largemouth bass *Micropterus salmoides*
Tilapia *Oreochromis niloticus*

Marine non-salmonids:

kona kampachi *Seriola rivoliana*
southern flounder *Paralichthys lethostigma*

Tropical (Aquarium) Species:

albino cory catfish *Corydoras aeneus*
angelfish *Pterophyllum scalare*
Cichlidae spp.
longfin danios *Brachydanio rerio*
neon tetras *Paracheirodon innesi*
swordtail *Xiphophorus helleri*