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Determination of Dose Rate of Florfenicol in Feed for Control of Mortality in Channel Catfish *Ictalurus punctatus* (Rafinesque) Infected with *Edwardsiella ictaluri*, Etiological Agent of Enteric Septicemia

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Abstract.—A dose titration study was conducted to determine the appropriate dosage of florfenicol in feed to control mortality in channel catfish *Ictalurus punctatus* associated with enteric septicemia of catfish caused by *Edwardsiella ictaluri*. Six tanks (20 fish/tank) were assigned to each of the following treatment: 1) not challenged with *E. ictaluri* and fed unmedicated feed; 2) challenged with *E. ictaluri* and fed unmedicated feed; 3) challenged and fed 5-mg florfenicol/kg body weight (kg bw); 4) challenged and fed 10-mg florfenicol/kg bw; or 5) challenged and fed 15-mg florfenicol/kg bw. Treatment was initiated the day after inoculation, and feed was administered by hand at 2.5% body weight for 10 consecutive days. Feeding activity was scored for all groups and was noted to be significantly less than the challenged, unmedicated group. Cumulative mortality in the challenged untreated group was 60%. The mortality in the unchallenged untreated group was 0%, and in the 5-, 10-, 15-mg florfenicol/kg bw group was 2.5%, 0.8%, and 2.5%, respectively. The mortality in each challenged, treated group and the non-challenged control group was significantly less than the challenged, unmedicated controls ($P < 0.0001$ for each contrast). There were no pairwise statistically significant contrasts among the florfenicol treated groups and the non-challenged control group. All 600 fish in the study were necropsied, cultured for bacteria, and examined by gross pathology. No specific lesions that could be associated with the antibiotic were observed. The efficacy of the 10 mg/kg dosage was confirmed in a separate dose confirmation study. In this study, fish in 30 tanks (20 fish/tank) were infected with *E. ictaluri* by immersion. Two days post-inoculation, fish in 15 tanks were hand-fed unmedicated feed, and 15 tanks were hand-fed medicated feed at a dosage of 10-mg florfenicol/kg bw at 2.5% body weight for 10 d. Feeding activity was scored and was noted to be significantly less than the challenged, unmedicated group. Cumulative mortality in the florfenicol group (14%) was significantly less than cumulative mortality in the untreated group (87.3%) ($P < 0.0001$). All 600 fish were submitted for bacterial culture, necropsied, and examined for gross pathology, and once again, no specific lesions that could be associated with the antibiotic were observed. The minimum inhibitory concentration of florfenicol against *E. ictaluri* in both studies was 0.25 ug/mL. Florfenicol was palatable, safe, and efficacious for control of mortality due to infection by *E. ictaluri* in catfish.

The 1997 National Animal Health Monitoring System (NAHMS) reported that bac-

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terial diseases account for approximately 70% of all catfish diseases observed in the catfish-producing states of the southeast US (USDA 1997). Of all bacteria cultured from catfish *Edwardsiella ictaluri*, the etiological agent of enteric septicemia of catfish (ESC) often causes mass mortalities in channel catfish *Ictalurus punctatus* (Thune 1991) and is considered to be the most important in terms of economic losses (MacMillan 1985). ESC typically occurs in the spring and fall months at water temperatures of 22–28 C, which provide optimal conditions for growth of *E. ictaluri* (Waltman and Shotts 1986). Currently, the only two antibiotics approved by the U.S. Food and Drug Administration for use with food fish are oxytetracycline and a sulfadimethoxine/ormetoprim combination. However, in catfish, oxytetracycline is only approved for use against *Aeromonas* and *Pseudomonas* infections, and bacterial resistance to both antibiotics has been reported (Johnson 1991; Plumb et al. 1995). In addition, palatability problems have been reported with sulfadimethoxine/ormetoprim combinations (Poe and Wilson 1989). Until recently, oxytetracycline feed was manufactured only as a sinking pellet, making it difficult for farmers to gauge feeding activity. Although a floating oxytetracycline feed has recently been developed, availability is limited. These problems point out the need for new antimicrobial drugs for the control of bacterial diseases in fish.

Florfenicol ([R-(R*,S*)]-2,2-dichloro-N-[1-(fluoromethyl)-2-hydroxy-2-[4-(methylsulfonyl)phenyl]ethyl-acetamide), is a fluorinated derivative of thiamphenicol, a chloramphenicol (CHPC) analogue that is approved for use in cattle in the US. Unlike CHPC, florfenicol (FFC) produces no toxic side effects in people, such as aplastic anemia and bone marrow suppression. Toxicosis has been eliminated by replacing the para NO_2 group on the CHPC molecule (believed to be responsible for the adverse effects) by a methyl-sulfonyl group in FFC (Nagabhusahan et al. 1992).

Most bacteria that are resistant to CHPC and thiamphenicol are sensitive to FFC (Cannon et al. 1990). Furthermore, FFC has been shown to be efficacious against a number of fish pathogens, including *Aeromonas salmonicida* and *Vibrio salmonicida* (Fukui et al. 1987; Inglis and Richards 1991; Samuelson et al. 1998; Nordmo et al. 1998; Bruun et al. 2000; Schmidt et al. 2000), and efficacy against *E. ictaluri* in vitro has also recently been demonstrated (McGinnis et al. 2003). FFC is currently approved for use in aquaculture to control susceptible bacterial infections in Japan (yellowtail *Seriola quinqueradiata*, red sea bream *Pagellus bogaraveo*, coho salmon *Oncorhynchus kisutch*, horse mackerel *Trachurus spp.*, rainbow trout *O. mykiss*, sweetfish *Plecoglossus altivelis*, tilapia *Oreochromis spp.*, and eel *Anguilla japonica*), South Korea (yellowtail and eel), Norway (Atlantic salmon *Salmo salar*), Canada (Atlantic salmon), and the UK (Atlantic salmon).

Efficacy studies evaluating FFC are limited. The results of a range-finding study on the therapeutic effects of FFC on experimentally induced ESC in channel catfish were evaluated and reported in a previous trial (Gaunt et al. 2003). FFC was found to be highly effective as a therapeutic agent, but determination of the optimum dose level was not evaluated. To consider approval of FFC for usage in catfish, the U.S. Food & Drug Administration Center for Veterinary Medicine required justification of the dosage using a dose titration and a dose confirmation study. Reported here are the results of two individual studies: 1) a dose titration study that determined a narrow dose range of FFC-medicated feed for the control of mortality in catfish associated with ESC; and 2) a dose confirmation study that verified the FFC dosage determined from the dose titration study.

Materials and Methods

Experimental Design—Dose Titration Study

Six rows of five tanks of fish (30 tanks total) were assigned by restricted randomi-

zation such that each row had one tank allotted to each of five treatment groups: Treatment groups included: 1) not challenged with *E. ictaluri* and fed unmedicated feed; 2) challenged with *E. ictaluri* and fed unmedicated feed; 3) challenged and fed 5-mg FFC/kg bw; 4) challenged and fed 10-mg FFC/kg bw; and 5) challenged and fed 15-mg FFC/kg bw. Twenty channel catfish fingerlings were counted into each tank in an undetermined ratio of males and females and acclimated to experimental conditions for 10 d. During acclimation fish were fed a commercial diet once per day at an estimated rate of 2.5% of their body weight, with the exception that fish were not fed 24 h before the bacterial challenge. On Day 0, fish from each tank were weighed, then challenged with *E. ictaluri* by immersion. Treatments were initiated the day after inoculation. Fish were hand fed treatments one time per day at 2.5% of their body weight for 10 consecutive d (Day 1–10). The primary response variable was mortality. When dead fish were removed from a tank, the quantity of feed administered to the tank was reduced proportionally to the decrease in tank biomass (based on mean fish weight). During the 14-d observation period after treatment, fish were fed commercial (non-medicated) catfish feed at a rate of 2.5% of their body weight. At the end of the study (Day 25), all surviving fish were removed from each aquarium, counted, and weighed as a group. Surviving fish were then euthanized and submitted for microbiologic and pathologic evaluation.

Experimental Design—Dose Confirmation Study

Thirty tanks each containing 20 channel catfish fingerlings were randomly allocated such that 15 tanks were assigned to each of the two treatment groups: 1) challenged with *E. ictaluri* and fed unmedicated feed; and 2) challenged and fed 10-mg FFC/kg bw. Fish were acclimated to experimental conditions for 21 d and challenged and fed as described above in the dose titration study. Fish were fed commercial feed on Day 1, and treatment was delayed until 2 d after immersion. Fish were hand fed to provide 0- or 10-mg FFC/kg of fish daily for 10 consecutive d (Days 2–11). Fish were also fed and monitored during the 14-d observation period after treatment as described above. The primary response variable was mortality. At the end of the study (Day 26), all surviving fish were removed from each tank, counted, and weighed as a group. Surviving fish were then euthanized and submitted for microbiologic and pathologic evaluation.

Fish

Six-hundred laboratory-reared 4–5-mo-old channel catfish fingerlings with no known history of exposure to *E. ictaluri* were used for each study. Fish were obtained from the Thad Cochran National Warmwater Aquaculture Center Aquaria Building in Stoneville, Mississippi for the dose titration study and from the USDA Catfish Genetics Research Unit in Stoneville, Mississippi for the dose confirmation study. All fish were considered naïve because they had never been exposed to an *E. ictaluri* epizootic. Serum titers to *E. ictaluri* were determined in 50 non-experimental fish from each source by a modified agglutination assay (Conrath 1972), and results were negative. Throughout all phases of both studies, fish were monitored for possible adverse events such as low dissolved oxygen or unexpected mortality. No adverse events were noted in either study.

Environmental Parameters

Thirty glass tanks (80 L) supplied with well water (single pass flow-through) were used for each study. During acclimation, fingerlings were fed a floating commercial catfish feed. During the entire study period, fish were fed daily at 2.5% of their body weight once daily, and their feed consumption was recorded. Water quality parameters measured twice weekly included temperature, pH, total ammonia, chloride, and ni-

trite. Water alkalinity and hardness were measured once prior to each study. Water supply to the tanks was analyzed for lead, copper, organochlorines, and organophosphates prior to the dose titration study. Overall water quality was excellent throughout the studies, and within acceptable limits for the maintenance of channel catfish fingerlings. No harmful levels of organophosphates, organochlorines, or metals were found in the water that would interfere with the husbandry of the channel catfish. The mean water flow rate to the tanks was 533 mL/min. The photoperiod was 12-h light and 12-h dark.

Preparation of Feed

Commercial catfish feed containing 32% crude protein (SF Services, Inc., North Little Rock, Arkansas, USA) and 36% crude protein (Clover Brand, Farmland Industries, Inc., Kansas City, Missouri, USA) were used for the dose titration study and dose confirmation study, respectively. These feeds were ground and pelleted according to a method previously described (Li et al. 1993). For medicated feeds, FFC Aquaculture Premix (Aquaflor®; 50% Type A Medicated Article), provided by Schering-Plough Animal Health Corporation (SPAH, Union, New Jersey, USA), was mixed with the dry ground feed then pelleted. For the dose titration study, feed containing 0-, 200-, 400-, and 600-mg FFC/kg feed was prepared and manufactured in order of increasing dose rates. For the dose confirmation study, feed containing 0- and 400-mg FFC/kg feed was prepared and the unmedicated feed was manufactured first. Feed was stored in a monitored refrigerator at 4 C.

Analysis of Medicated Feeds

Xenos Laboratories (Ottawa, Canada) analyzed all feeds for FFC concentration. Briefly, FFC was extracted from 10 g of medicated catfish feed in 100 mL of 50/50 acetonitrile/water by two cycles of sonication (5 min at 50 C) and shaking (10 min). The extract was allowed to settle before decanting into a centrifuge tube. After centrifugation, the extract was transferred to an EnviCarb cartridge for sample clean-up. The cartridge was rinsed with the extraction solvent, and the eluate was collected and analyzed by reversed-phase high-pressure liquid chromatography. The chromatographic system employed a Nucleosil ODS column (5-μm particle size, 250 × 4.6 mm) with sodium acetate/acetonitrile (2:1, v/v), pH 4.4, mobile phase at a flow rate of 0.8 mL/min, and ultraviolet detection at 225 nm.

The concentration of FFC in the 200-, 400-, and 600-ppm feed for the dose titration study was 182 ppm (91% of nominal), 380 ppm (95% of nominal) and 555 ppm (92.5% of nominal), respectively. In the dose confirmation study, the concentration of FFC in the 400-ppm medicated feed was 421.5 ppm (105.4% of nominal). FFC levels in the non-medicated feed were all below the limit of quantitation. Woodson-Tenent Laboratories (Memphis, Tennessee, USA) analyzed all feeds for oxytetracycline or sulfadimethoxine and ormetoprim residues, and SGS Agricultural Services (Memphis, Tennessee, USA) analyzed all feeds for organophosphates or organochlorine residues. None of these potential contaminants were found to be present at levels that would adversely affect the outcome of the studies.

Preparation of E. ictaluri *Inoculum and Challenge of Fish*

The *E. ictaluri* isolate (S94–887) was obtained from a channel catfish submitted to the College of Veterinary Medicine Fish Diagnostic Laboratory, Stoneville, Mississippi, USA, during a natural outbreak of ESC. The isolate was identified by biochemical characteristics using a biochemical test kit according to manufacturer's instructions (BBL Crystal, Cockeysville, Maryland, USA). Using 30-μg FFC discs supplied by SPAH (Union, New Jersey, USA) the Kirby-Bauer zone of inhibition (Bauer et al. 1966) was measured on a

Mueller-Hinton agar plate with 5% sheep blood (MHA) uniformly streaked with *E. ictaluri* and determined to be 51 mm. To confirm and enhance pathogenicity, the isolate was placed in Brain Heart Infusion (BHI) broth and incubated at 25 C for greater than 24 h. After incubation, 0.1 mL of the *E. ictaluri* in BHI suspension was injected intracoelomically to each of four passage fish. These inoculated fish died 1–4 d post inoculation. Dead fish were submitted for gross pathology examination and bacterial culture. Brains and posterior kidneys from these inoculated fish were cultured on Mueller-Hinton agar with 5% sheep blood and incubated at 25 C. To produce the inoculum, two to three recovered colonies were placed into each of four test tubes for the dose titration study and five tubes for the dose confirmation study containing 10-mL BHI broth and incubated at 25 C for 24 h. Each 10-mL tube was decanted into 1,000-mL BHI broth and incubated at 25 C for 24 h. After incubation, 5 mL of inoculated broth was removed from each liter and serially diluted to perform colony counts of bacteria as well as purity checks. In a method previously described, fish were exposed to *E. ictaluri* by immersion challenge (Wise et al. 1997). Water flow to all 30 aquaria was turned off, and the 80-L tanks were drained to one-quarter full to give an approximate volume of 25 L. In the dose titration study, the 4 L of BHI broth were pooled and in the dose confirmation study, the 5 L of BHI broth were pooled. One-hundred mL of the inoculum was placed into each of the partially drained tanks. The number of colony forming units (cfu)/mL to inoculate the tanks was 23×10^7 cfu/mL for the dose titration and 23.5×10^7 cfu/mL for the dose confirmation study. The challenged fingerlings were exposed for 2 h to a calculated concentration of *E. ictaluri* at 8.8×10^5 cfu/mL and 9.5×10^5cfu/mL for the dose titration and dose confirmation studies, respectively. After the exposure, normal water flow was restored and the tanks were refilled.

Microbial Methods

Cultures for bacterial pathogens were collected from the brain and posterior kidney of each fish and incubated at 25 C for 2 d. *E. ictaluri* was identified on the basis of biochemical results (Shotts and Bullock 1975; Hawke et al.1981) through tests supplied by Difco (Becton Dickinson and Company, Cockeysville, Maryland, USA) and BBL Crystal (Cockeysville, Maryland, USA). Kirby-Bauer zones of inhibition were performed for all identified *E. ictaluri* in each study to assess sensitivity to FFC according to standard methods. Colonies were removed from blood plates and suspended in broth to the density of a 0.5 McFarland turbidity standard. After blood agar plates were evenly inoculated with a swab, a 30-μg FFC sensitivity disc was placed in the middle of the plate. Plates were incubated for 2 d at 25 C; and the diameter of the zones of complete inhibition of *E. ictaluri* growth (including the diameter of the disk) were measured. The diameters of the zones ranged from 31 to 33 mm in the dose titration study and from 32 to 41 mm in the dose confirmation study. The minimum inhibitory concentration (MIC) was determined for the inoculum bacteria prior to inoculation and for selected cultures from each study. The colonies were removed from the blood agar plates and suspended in BHI to the density of a 0.5 McFarland Turbidity Standard. One μL of each bacterial isolate was inoculated on the surface of blood agar plates prepared with concentrations of FFC ranging from 0.06 to 16 ug/mL. Plates were incubated at 25 C for 2 d and observed to determine which concentration completely inhibited growth of *E. ictaluri* (NCCLS 1999). The MIC for the inoculum bacteria and for all isolates tested from each study was 0.25 μg/mL.

Feeding, Behavioral Observations

Feeding activity and morbidity were assessed separately using the following pro-

cedures: Fish were hand fed one time per day, and feeding activity was recorded. A numerical score of 2 was assigned to a tank if approximately 50–100% food was consumed. A numerical score of 1 was assigned to a tank if less than half the food was consumed. A numerical score of 0 was assigned to a tank if no food consumption was detected. Fish were visually observed once daily for behavior changes and clinical signs indicative of ESC. Because a fish scored as morbid could either spontaneously recover, be scored as morbid on subsequent time points, or die, and since individual fish were not identified, no overall summations or statistical analysis of morbidity could be accurately determined.

Gross Necropsy

All morbid and dead fish were removed and submitted for necropsy. Morbid fish were euthanized with an overdose of MS-222 (Tricaine-S, Western Chemical, Inc., Ferndale, Washington, USA). Post-mortem examination of the fish included an inspection of the skin, fins, mouth, eyes, gill, and coelomic viscera.

Statistical Analysis

At the termination of each study, data were examined for statistically significant differences ($P < 0.05$) in fish mortality, weight change, and positive tissue cultures for *E. ictaluri*. In the dose titration study, comparisons were made between the challenged untreated group, the unchallenged group, and the challenged treated groups, and, between the low and the high dose groups. In the dose confirmation study, comparisons were made between the untreated group and the treated group.

The analysis of mortality data (binomial data) followed the protocol specified Logistic Regression using a General Linear Mixed Model (SAS %GLIMMIX, SAS Institute, Inc., Cary, North Carolina, USA) with fish nested within tanks and tanks nested within treatment (binomial error with default logit link). The dose titration study also included a linear-plateau analysis to evaluate dose response.

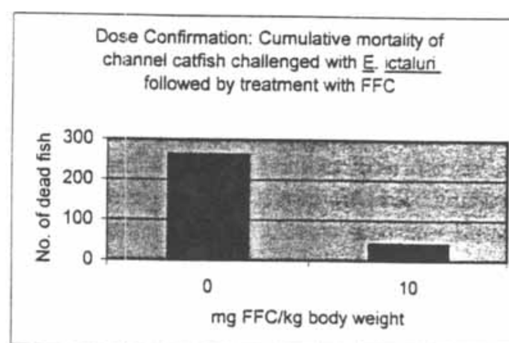

FIGURE 1. *Dose Titration Study: Cumulative mortality of* E. ictaluri*-exposed channel catfish treated with 0-, 5-, 10-, or 15-mg FFC/kg body weight for 10 d in medicated feed. Treatment was initiated the day after exposure to* E. ictaluri.

Fish were weighed by tank at the day of challenge and the number of fish in the tank was factored to yield a tank average fish weight. An analysis of variance was run to demonstrate no pre-challenge differences in average fish weight. The tanks were again weighed at the conclusion of the study, but no statistical tabulations are provided because few of the challenged untreated control fish survived. Statistical significance was declared at $P \leq 0.05$ and preliminary significance at $0.05 < P < 0.10$. Two-tailed tests were run prior to challenge and administration of medicated feed, and one-tailed tests were run post-treatment to test the strict inequality that treatment with florfenicol was more efficacious than non-medicated feed.

Results

Dose Titration Study

Following inoculation of the tanks (Day 0), the first mortalities were identified in a challenged, non-medicated control tank on Day 5. Mortalities were seen in this group from Day 5 to Day 18, with the majority occurring on Days 6 (10.8%, 13/120), 7 (12.5%, 15/120), and 8 (8.3%, 10/120). The mean cumulative percent mortality (Fig. 1) in this group was 60% ± 15.5% fish, with

the cumulative tank mortalities ranging from 45% (9/20 fish) to 85% (17/20). The non-challenged, non-medicated control group had one mortality (0.8%, 1/120). The low dose medicated group (5-mg FFC/kg bw) had one mortality on each of Days 4, 6, and 15 (0.8%, 1/120, for each occurrence) and two mortalities on Day 25 (day of termination) (1.6% 2/120), for a cumulative mortality rate of 4.2% (5/120). The middle dose medicated group (10 mg FFC/kg bw) had only one mortality occurring on Day 8 (0.8%, 1/120). The high dose medicated group (15 mg FFC/kg bw) had one mortality on each of Days 4, 7, and 11 (0.8%, 1/120, for each occurrence), for a cumulative mortality rate of 2.5% (3/120) (Fig.1). The mortality in each challenged, treated group and the non-challenged control group was significantly less than the challenged unmedicated control group ($P < 0.0001$ for each contrast). There were no pairwise statistically significant contrasts among the florfenicol treated groups and the non-challenged control group.

In the challenged, unmedicated control group, *E. ictaluri* was recovered from 72.3% (86/119) of the fish. In the unchallenged, unmedicated control group, *E. ictaluri* was recovered from none of the fish. In the florfenicol treated fish, *E. ictaluri* was recovered from 2.5% (3/118) of fish in the 5-mg FFC/kg bw treated group, 1.7% (2/120) of fish in the 10-mg FFC/kg treated group, and 3.3% (4/120) of fish in the 15-mg FFC/kg bw group. Bacterial colonies characteristic of *E. ictaluri* were cultured from 100% (78/78) of dead fish recovered from 13/30 tanks.

Bacteria cultured from survivors included one *E. ictaluri* positive fish from the 10-mg FFC/kg bw group and one positive fish from the 15-mg FFC/kg bw group. Organisms were confirmed as *E. ictaluri* on the basis of biochemical test results (Shotts and Bullock 1975; Hawke et al. 1981). The 17 tanks that did not yield mortalities (six of the unchallenged tanks, three of the 5-mg FFC/kg bw, five of the 10-mg FFC/kg bw, and three of the 15-mg FFC/kg bw) had no cultures positive for *E. ictaluri* with the exception of one fish recovered from a tank that received 10-mg FFC/kg bw.

Postmortem examination of mortalities and/or *E. ictaluri* positive fish revealed external lesions including inflammation through the sutra fontanel of the skull (commonly known as "hole in the head"), hemorrhages on the skin and fins, and exophthalmia compatible with ESC (Baldwin and Newton 1993). Internal lesions included gastrointestinal and liver hemorrhages, congested spleens, ascites, and swollen kidneys. These lesions were observed in 81% (77/95) of fish determined positive for *E. ictaluri* (including cultures from mortalities and euthanized fish) in the dose titration study. The prevalence of these lesions was increased in the untreated, challenged group compared to the treated group. In the unchallenged group, no gross lesions were observed.

Mean fish weight at the time of challenge ranged from 4.45 g to 7.30 g and the mean fish weight for survivors at the end of the study (Day 25) ranged from 6.5 g to 9.84 g. Feeding activity in the unchallenged, unmedicated fish was graded as 2 throughout the study. Feeding activity of all the FFC medicated tanks was 2 from Days 1 through 24 with the exception of Day 18. On this day, one of the 15-mg FFC/kg bw tanks had a score of 1. The mean feeding activity of the challenged, unmedicated fish ranged from 0.33 to 1.67 from Day 3 to 13. From Day 3 onward, the average feeding score did not return to 2 with the exception of Day 23.

Dose Confirmation Study

Following inoculation of the tanks (Day 0), the first mortalities were identified in a control tank on Day 3. The majority of the mortalities were seen in this group from Day 5 to Day 11 (86%, 258/300), with the mode occurring on Day 7 (30.7%, 92/300). The mean cumulative percent mortality (Fig. 2) in the untreated control group was

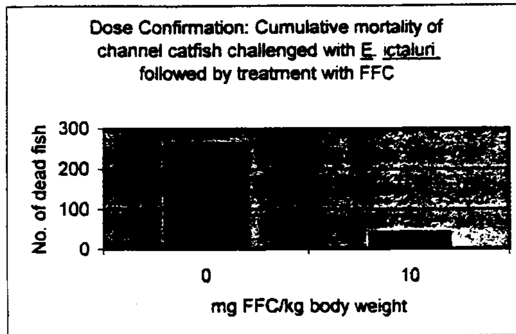

FIGURE 2. *Dose Confirmation Study: Cumulative mortality of channel catfish that were challenged with* E. ictaluri *followed by treatment with either 0- or 10-mg FFC/kg body weight for 10 d in medicated feed. Treatment was initiated 2 d after exposure to* E. ictaluri.

87.3% ± 11.3%, with individual tank mortalities ranging from 60% (12/20 fish) to 100% (20/20). The majority of the FFC medicated group (10-mg FFC/kg bw) mortalities occurred from Day 5 to Day 7 (13%, 39/300) with the mode occurring on Day 6 (8.7%, 26/300). The cumulative mortality for the FFC medicated group was 14% (42/300) with mortality of individual tanks ranging from 0 to 75%. Mortality in the treated group was significantly less than the control group ($P < 0.0001$). In the control group, *E. ictaluri* was recovered from 251 of 300 (83.7%) fish. For the florfenicol treated fish, *E. ictaluri* was recovered from 28 of 300 (9.3%) fish.

Bacterial colonies characteristic of *E. ictaluri* were cultured from 94% (279/304) of dead fish recovered from 26 of 30 tanks, and these were confirmed as *E. ictaluri* on the basis of biochemical test results (Hawke et al.1981; Shotts and Bullock 1975). *E. ictaluri* was not cultured in any of the survivors. The four tanks that did not yield mortalities (all FFC-treated) had no cultures positive for *E. ictaluri.*

Gross lesions as described in the dose titration study were seen in 88% (268/304) of fish determined positive for *E. ictaluri* in the dose confirmation study. The incidence of these lesions was increased in the untreated group compared to the treated group. No lesions indicative of any concurrent diseases were observed in either study.

Mean fish weight at the time of challenge ranged from 6.0 g to 10.56 g and the mean fish weight of survivors at the end of the study (day 25) ranged from 11.0 g to 17.5 g. The mean feeding activity score of FFC-medicated fish ranged from 1.67 to 2 during treatment. The least feeding activity was seen on Day 4, at which time five of 15 tanks had a feeding activity of 1 and 10 of 15 tanks had a feeding activity of 2. From Day 6 onward, at least 14 of 15 FFC medicated tanks maintained feeding scores of 2. On Day 4 when the FFC-treated group reached the nadir of its feeding activity at 1.67, the control group also reached its lowest average feeding score of 0.13. The feeding activity of the majority of the unmedicated fish was observed to be either 1 or 0 on Day 3 through Day 11. By Day 9, all fish had died in three of the control tanks. By Day 16, the feeding response improved in the remaining 12 control tanks to either 1 or 2. By Day 20, all remaining control tanks returned to a feeding response score of 2.

Discussion

In both studies, initiation of FFC treatment after exposure to *E. ictaluri* resulted in significantly higher survival rates as compared to the unmedicated groups. In the dose titration study, cumulative mortality ranged from 0.8% to 4.2% in FFC-treated fish as compared to 60% in the unmedicated group. In the dose confirmation study, mortality was 14% in the FFC-treated group as compared to 87.3% in the unmedicated group. The somewhat higher percentage of mortalities in the medicated group in the dose confirmation study may have occurred because treatment was initiated 2 d after inoculation instead of 1 d as in the dose titration study. Another possible explanation for this variance could be the different source of the two groups of fish, as the fish

in the dose titration study may have been less susceptible to this pathogen.

Percent mortalities in untreated, challenged, and medicated challenged fish were comparable to previous reports. The cumulative mortality of untreated fish were 60% and 87.3% for the FFC dose titration study and the FFC dose confirmation study, respectively. In a previous tank study on the efficacy of antibiotics to treat ESC, the cumulative mortality of untreated fish was 63% (Wise et al. 1997). When sarafloxacin was used to treat ESC-infected fish 4 h post-immersion, the mean mortality rate for treated fish was 2.8% at dosages of both 10 mg/kg bw and of 14 mg/kg (Thune and Johnson 1992). In another ESC tank challenge using dosages of 10-mg sarafloxacin/kg bw for 5 d, the mortality was 17% in the first trial and 5% in the second trial (Plumb and Vinitnantharat 1990). Fish were treated 24 h post-inoculation.

A factor that may have contributed to the high survival rate in FFC-medicated fish in both studies was the feeding rate of 2.5% body weight, which distributed the medication over a larger number of feed pellets and optimized intake of FFC. Rates of feeding medicated feed to *E. ictaluri* infected fish are often decreased to 1% because of formulation characteristics of the available antimicrobial medicated feeds and depressed appetite. However, in previous experiments, the number of surviving fish was significantly higher when feeding rates of Romet® medicated feed were increased from 1% to 3% (Johnson and Smith 1994). Ponds with higher feeding rates had more pellets available to achieve the same medication level, and this allowed medication of more fish including less aggressive eaters.

By Day 2 of the dose confirmation study (the third d after exposure), many of the fish (including fish in treated group) began to experience a diminution in appetite. A few of the medicated and many of the challenged unmedicated fish were overcome by the infection and succumbed. However, by Day 6, the mean feeding activity of the treated tanks had returned to the pre-study level with the exception of one tank on Day 10 and another tank on Day 15. In the dose titration study, only the untreated challenged groups experienced a diminution in feed beginning Day 3 (the third d after exposure). However, the treated groups in both studies showed significant decreases in mortality when compared to the untreated groups, demonstrating that oral treatment with FFC offered *E. ictaluri*-challenged fish significant protection against mortality. These results suggest that FFC was well absorbed by the gastrointestinal tract, and they are in agreement with a previous report in which the use of FFC in pelleted feed was a highly effective method of medicating fish (Samuelson et al. 1998).

Although an earlier study demonstrated that a 10-mg FFC/kg bw dose rate for 5 d significantly decreased mortality (Gaunt et al. 2003), and the dose titration study demonstrated that 5-mg FFC/kg bw for 10 d also afforded significant protection, a 10-d 10-mg FFC/kg bw regime was chosen as the treatment in the dose confirmation study. A previous report describing *E. ictaluri* infection in channel catfish after immersion exposure demonstrated that mortality was accompanied by shedding of viable bacteria in the water without a decrease in the numbers of cfu/mL of water until 6 d after exposure (Wise et al. 1997). This long shedding time suggested that the 10-d feeding regime of FFC would offer more protection against *E. ictaluri* infection than a 5-d regime. Although the dose titration study showed no significant differences in mortality between the 5- and 10-mg FFC/kg bw groups, the higher dosage of FFC was selected as the dose confirmation treatment. Fish have hierarchies of feeding, and more aggressive fish receive higher dosages of medication than less aggressive feeders (McCarthy et al. 1992). Therefore, a higher dosage of FFC was chosen to ensure adequate intake of medication to all fish.

Observations verified that between 84–

100% of the maximum feeding scores for the medicated tanks was achieved. Based on the statistical outcome of the dose confirmation study, the dose rate of 10 mg/kg for 10 d was adequate for the fingerling fish to consume ample medication.

No specific gross pathology lesions that could be associated with FFC were observed in either the dose titration or dose confirmation study. This is in agreement with previous range-finding studies in which no gross or histopathologic FFC-related lesions were observed among 640 fish in efficacy and tolerance studies with dose rates ranging from 10–40 mg FFC/kg bw, and 10–100 mg FFC/kg bw, respectively (Gaunt et al. 2003).

The low MIC for FFC (0.25 μg/mL) indicated potent in vitro antimicrobial activity against the *E. ictaluri* isolate tested. This value agrees with other reported FFC MIC values which range from 0.3 to 1.6 μg/mL for other fish pathogenic bacteria (Fukui et al. 1987; Inglis and Richards 1991; Martinsen et al. 1993; Bruun et al. 2000; Schmidt et al. 2000).

In conclusion, FFC at a daily dose rate of 10-mg FFC/kg bw of fish for 10 consecutive d was effective in preventing mortality from *E. ictaluri* in channel catfish fingerlings when feeding was initiated 1–2 d post-infection.

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