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Preliminary Assessment of the Tolerance and Efficacy of Florfenicol against *Edwardsiella ictaluri* Administered in Feed to Channel Catfish

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Abstract.—A tolerance study was conducted to determine the palatability of florfenicol to channel catfish *Ictalurus punctatus*. Four tanks of fish (20 fish/tank) were assigned to each of five treatments distinguished by the amount of florfenicol given in feed per kilogram of body weight, namely, 0, 10, 20, 40, or 100 mg. Fish were fed at a rate of 2.5% of body weight per day for 10 consecutive days. On day 11, all surviving fish were euthanized, counted, and weighed as a group. Florfenicol-medicated feed was palatable to fish at doses of 10, 20, 40, and 100 mg for 10 consecutive days.

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All 400 fish were necropsied and examined by histopathology, and no treatment-related changes were observed. In a separate exploratory efficacy study, four tanks (20 fish/tank) were assigned to each of the following treatments: (1) not challenged with *Edwardsiella ictaluri* and fed unmedicated feed, (2) challenged with *E. ictaluri* and fed unmedicated feed, (3) challenged with *E. ictaluri* and fed florfenicol at 10 mg per kilogram of body weight, (4) challenged and fed florfenicol at 20 mg/kg, and (5) challenged and fed florfenicol at 40 mg/kg. Treatment was initiated the day after inoculation, and feed was administered by hand for five consecutive days at 2.5% of body weight. The cumulative mortality observed over a 17-d period in treatment groups 1–5 was 2.5, 57.5, 0, 1.25, and 1.25%, respectively. All 400 fish were necropsied and examined by histopathology. The results indicate that florfenicol was effective in controlling mortality from enteric septicemia of catfish with no adverse treatment-related tissue changes. Florfenicol was palatable, safe, and efficacious in controlling mortality due to infection by *E. ictaluri* in channel catfish.

Edwardsiella ictaluri, the etiological agent of enteric septicemia of catfish (ESC) causes mass mortalities in channel catfish *Ictalurus punctatus* (Thune 1991). Enteric septicemia of catfish occurs in the spring and fall months when water temperatures are 20–28°C and conditions are optimal for the growth of *E. ictaluri* (Miyazaki and Plumb 1985; Francis-Floyd et al. 1987). In 1986 *E. ictaluri* was reported to be susceptible to a variety of antibacterial agents, including the only two antibiotics currently approved for treatment of ESC in catfish, the potentiated sulfonamide Romet (sulfadimethoxine−ormetoprim), and Terramycin (oxytetracycline; Waltman and Shotts 1986). Resistance to both antibiotics has been reported (Johnson 1991; Plumb et al. 1995) after less than 10 years of use in catfish aquaculture. The fluoroquinolone sarafloxacin was the last drug developed for treatment of *E. ictaluri* infections in channel catfish (Johnson and Smith 1992; Thune and Johnson 1992; Meade et al. 1993). However, in 1994 the U.S. Food and Drug Administration placed a moratorium on all nonhuman uses of fluoroquinolones, and as a result sarafloxacin was not registered for use in aquaculture in the United States. No other drugs for the control of bacterial diseases in fish have been developed in the United States since that time.

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 analog. It is approved for use in cattle, swine, and poultry in the United States, Europe, and several other countries. However, florfenicol is not associated with the aplastic anemia and bone marrow suppression reported in humans after treatment with chloramphenicol because the para-NO_2 group on the chloramphenicol molecule believed to be responsible for the adverse effects has been replaced by a methyl sulfonyl group (Nagabhusahan et al. 1992). The structure of florfenicol is shown in Figure 1.

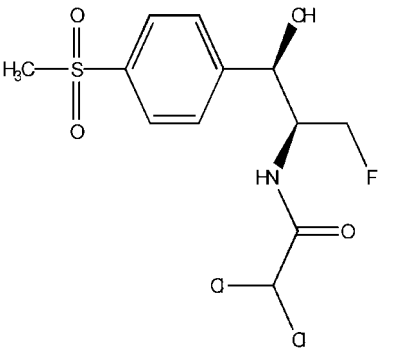

FIGURE 1.—Chemical structure of active florfenicol.

Bacteria that are resistant to chloramphenicol and thiamphenicol have been shown to be sensitive to florfenicol (Cannon et al. 1990); florfenicol has also been shown to be efficacious against a number of fish pathogens (Fukui et al. 1987; Inglis and Richards 1991; Nordmo et al. 1998; Samuelson et al. 1998). Its efficacy against *E. ictaluri* in vitro has recently been demonstrated (McGinnis et al. 2003). Florfenicol has been shown to have high bioavailability and a significant degree of tissue penetration, including penetration of the brain in Atlantic salmon *Salmo salar* (Martinsen et al. 1993; Horsberg et al. 1994). It is presently approved for aquaculture to control susceptible bacterial diseases in Japan (yellowtail [also known as buri] *Seriola quinqueradiata*, red sea bream *Pagellus bogaraveo*, coho salmon *Oncorhynchus kisutch*, horse mackerel *Trachurus* spp., rainbow trout *O. mykiss*, sweetfish [also known as ayu] *Plecoglossus altivelis*, tilapia *Oreochromis* spp., and Japanese eel *Anguilla japonica*), South Korea (yellowtail and eel), Norway (salmon), Canada (salmon), and the United Kingdom (salmon).

In this article we report the results of two studies. The objective of the first study was to determine the tolerance of channel catfish for florfenicol-medicated feed as assessed by palatability and weight gain. The objective of the second was to

determine the appropriate dose of forfenicol in feed to control ESC. We also looked for florfenicol-induced lesions by assessing gross and histopathological tissue changes.

Methods

Fish.—Eight-hundred laboratory-reared, 5-month-old channel catfish fingerlings with no known exposure to *E. ictaluri* were obtained from the Mississippi State University College of Veterinary Medicine in Starkville. The fingerlings were counted and weighed in groups of 20 in an undetermined ratio of males and females. In the efficacy study, however, one treated tank (10 mg of florfenicol per kilogram of body weight) was inadvertently allotted 21 fish.

Fish environment and handling.—Twenty flow-through glass tanks (120 L) were used for each study. Unchlorinated well water was supplied to all tanks, and aeration was achieved utilizing compressed air from air stones. Water quality variables were measured once per week during each study, with mean values as follows: temperature in the efficacy study, 23.6°C; temperature in the tolerance study, 27.7°C; pH, 8.77; chloride, 338.9 mg/L; nitrite, 1.12 mg/L; total ammonia, 1.38 mg/L; alkalinity, 393.7 mg/L; and hardness, 393.7 mg/L. 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) was weighed and ground in a hammer mill (Holmes Model 250 Sample Crusher, Danville, Illinois). Dry ingredients, which included Florfenicol Aquaculture Premix (50% Type A Medicated Article) provided by Schering-Plough Animal Health Corporation (Union, New Jersey) for medicated feeds, were placed in a V-mixer (Patterson-Kelley Blend Master Model B, East Stroudsburg, Pennsylvania) and mixed for 20 min. For the efficacy study, feed containing 0, 400, 800, and 1,600 mg of florfenicol per kilogram of feed was prepared in order of increasing florfenicol concentration; for the tolerance study, feed containing 0, 400, 800, 1,600, and 4,000 mg was prepared. Mixed ingredients were placed in a bowl, moistened with 30% deionized water by weight, and blended in a food mixer (Hobart Model A200 Stand Mixer, Troy, Ohio). This moistened mixture was subsequently cold-extruded in a chopper (Hobart Model 4822) to produce 1/8-in pellets, which were dried in a forced air oven (Grieve Model 13-261-28A, Round Lake, Illinois) at 25°C for approximately 2 h. Feed was stored at 4°C in a monitored refrigerator until use.

Feeding and feeding behavior.—During acclimation, the fingerlings received floating commercial catfish feed containing 32% crude protein (SF Services) at a rate of 2.5% of their body weight once daily. Following acclimation, fish were fed experimental diets at 2.5% of their body weight daily, and their feeding behavior and feed consumption were recorded. After administration of the experimental diets, fish received control feed at 2.5% of body weight per day.

Fish were hand-fed and observed for feeding activity daily. A numerical score of 0 was recorded if no food consumption was detected. A score of 1 was recorded for a tank if less than 50% of the feed was consumed and a score of 2 if more than 50% of the feed was consumed. In the tolerance study, a palatability score was computed as the sum of the daily scores for each treatment group for the 10-d treatment period divided by four. Hence the maximum score for consumption of all feed was 20.

Analysis of medicated feeds.—The Xenos Division of Enviro-Test Laboratories (Nepean, Ottawa, Ontario) analyzed all feeds for florfenicol concentration. Briefly, florfenicol was extracted from the catfish feeds by sonicating them with a 1:1 mixture of water and acetonitrile. This procedure was repeated and the extract allowed to settle before it was decanted into a centrifuge tube. After centrifugation, the extract was transferred to an EnviCarb (Supelco, Bellefonte, Pennsylvania) cartridge, in which it was allowed to drain. The cartridge was then rinsed with the extraction solvent, and the eluants were collected and analyzed by high-performance liquid chromatography with ultraviolet detection.

The florfenicol was mixed homogenously in the ground feed, and analysis established that the florfenicol was stable in catfish feed produced on a laboratory scale. The concentrations of florfenicol in the nominal 400-, 800-, 1,600-, and 4,000-mg/L feeds were 397 (99.2%), 781 (97.6%), 1,594 (99.6%), and 3,883 mg/L (97.1%), respectively. Florfenicol levels in the nonmedicated feed were all below the limit of quantitation.

Necropsy and histopathological methods.—All morbid and dead fish were removed and submitted for necropsy. Morbid fish were euthanatized with an overdose of MS-222 (tricaine methanesulfonate, Western Chemicals, Inc., Ferndale, Washington). The postmortem examination of the fish included an inspection of the skin, fins, mouth, eyes, gills, and coelomic viscera. Tissues were collected in 10% solutions of buffered formalin and included

the liver, anterior and posterior kidneys, spleen, heart, gills, skin with muscle, brain, and gastrointestinal tract. Tissues from all channel catfish were processed with routine histological methods, embedded in paraffin, and stained with hematoxylin and eosin (Areechon and Plumb 1983). Microscopic examination was performed on all tissue sections collected from the necropsied catfish in each of the dose groups. The severity of the changes was graded on a scale of 0 to 3 (0 = no lesion, 1 = mild, 2 = moderate, and 3 = severe).

Experimental design for the tolerance trial.—Four tanks of channel catfish (20 fish/tank) were randomly assigned to each of five treatment groups defined according to the quantity of florfenicol in the feed, namely, 0, 10, 20, 40, and 100 mg per kilogram of body weight. Fish were acclimated for 6 d. Feed was withheld the next day (day 0 of the trial), when fish from each tank were weighed as a group in a tared bucket of water. Beginning on the day after weighing, fish were hand-fed (at the rate of 2.5% of body weight per day) feeds to provide the target levels of florfenicol for 10 consecutive days (days 1–10). Fish were observed daily for mortality, signs of toxicity, and feeding behavior. Any fish that died during the study were necropsied and processed for histological examination. All surviving fish were removed from the aquaria (day 11), counted, weighed as a group, euthanatized, necropsied, and processed for histological examination.

At the termination of the tolerance study, data were examined for statistically significant differences in weight gain by analysis of variance (ANOVA) using the GLM procedure in SAS (SAS Institute 1999). Day 0 was included in the model as a covariate, and differences between groups were deemed statistically significant at $P \leq 0.05$. Histopathologic data were not analyzed statistically due to the finding of few abnormalities.

Experimental design of the efficacy trial.—Four tanks of fish (20 fish/tank) were assigned according to a randomized complete-block design to each of five treatment groups: (1) not challenged with *E. ictaluri* and fed unmedicated feed, (2) challenged with *E. ictaluri* and fed unmedicated feed, (3) challenged and fed florfenicol at the rate of 10 mg per kilogram of body weight, (4) challenged and fed florfenicol at the rate of 20 mg per kilogram of body weight, and (5) challenged and fed florfenicol at the rate of 40 mg per kilogram of body weight. Fish were acclimated to the experimental conditions for 7 d and were not fed for 24 h before challenge. Prior to inoculation, fish from each tank were weighed as a group in a tared bucket of water. Tanks were inoculated on day 0, and treatments were initiated the day after inoculation. Fish were hand-fed (at the rate of 2.5% of body weight per day) to provide the target levels of florfenicol for five consecutive days (days 1–5). During the 17-d observation period after the treatment, fish were fed commercial catfish feed containing 32% crude protein at the rate of 2.5% of body weight. Fish were observed twice daily for mortality. Any fish that died during the study were necropsied, and isolation of *E. ictaluri* was attempted. After the removal of dead fish, the amount of feed offered to each tank was readjusted by multiplying 2.5% times the number of dead fish times the average body weight of a fish from the affected tank and subtracting this from the allotted feed. At the end of the study, all surviving fish were removed from the aquaria, counted, and weighed as a group. Surviving fish were euthanatized on day 23 and submitted for microbiologic and histopathologic evaluation.

The *E. ictaluri* isolate (S94-887) was obtained from a channel catfish that became infected during a natural ESC epizootic and that was submitted to the College of Veterinary Medicine Fish Diagnostic Laboratory, Stoneville, Mississippi, in 1994. The isolate was identified by biochemical characteristics (Hawke et al. 1981). Briefly, colonies were removed from Mueller–Hinton blood agar plates and suspended in a broth medium to the density of a 0.5 McFarland turbidity standard. One μL of the suspension was evenly inoculated on the surface of blood agar plates and incubated at 25°C for 2 d.

To produce the inoculum, one colony was placed into each of three test tubes containing 10 mL of brain–heart-infusion (BHI) broth and incubated at 25°C for 24 h. The 24-h cultures were used to inoculate three 2-L flasks, each containing 1,000 mL of BHI broth, that were cultured at 25°C for 24 h. After incubation, 5 mL of inoculated broth was removed from each liter and bacterial densities were estimated by serial dilution and plate counts. Prior to the initiation of the efficacy study, the minimum inhibitory concentration (MIC) for florfenicol was determined to be 0.25 μg/mL for this isolate, and the Kirby–Bauer zone of inhibition was determined to be 51 mm (National Committee for Clinical Laboratory Standards 1990; McGinnis et al. 2003).

Fish were exposed to *E. ictaluri* by immersion challenge (Ciembor et al. 1995). Water flow to all 20 aquaria was turned off, and the 120-L tanks

were drained so that they were approximately one-fourth full (approximate volume, 30 L). One hundred mL of the inoculum was added to each challenged tank. One hundred mL of sterile BHI broth was added to each nonchallenged tank. The challenged fingerlings were exposed to a calculated concentration of 4.3×10^6 colony-forming units (CFU) of *E. ictaluri* per milliliter for 2 h. After the 2-h exposure, normal water flow was resumed and the tanks were refilled.

Fish from the efficacy trial were cultured (brain and posterior kidney) for bacterial pathogens. Isolations were made on Mueller–Hinton agar containing 5% sheep's blood. Plates were then incubated at 25°C for 2 d. Cultures that yielded the small, white, punctate, weakly β-hemolytic colonies characteristic of *E. ictaluri* were tested for oxidase and indole. Negative results for oxidase and indole indicated the presence of *E. ictaluri*. On selected isolates, *E. ictaluri* was identified biochemically by means of a Minitek System (Becton-Dickinson & Co., Sparks, Maryland).

Zones of inhibition were determined by the Kirby–Bauer method as outlined in the National Committee for Clinical Laboratory Standards performance standards guideline (National Committee for Clinical Laboratory Standards 1990; Bauer et al. 1966). Bacterial growth from the reisolated cultures was removed with a sterile cotton swab and suspended in BHI media to the density of a 0.5 McFarland barium sulfate turbidity standard. Plates were streaked with the swab and rotated to provide an evenly distributed inoculum of *E. ictaluri*. A sensitivity disk impregnated with 30 μg of florfenicol was placed on each plate with sterile forceps. Plates were then incubated for at least 2 d at 25 ± 2°C until the zone of inhibition was clearly defined. The zone of bacterial growth inhibition (including disk diameter) was measured to the nearest millimeter with a certified caliper. Control plates containing *Escherichia coli* ATCC (American Type Culture Collection) 25922 were incubated at 25 ± 2°C and 35 ± 2°C and observed in accordance with National Committee for Clinical Laboratory Standards guidelines.

At the termination of the efficacy study, data were examined for statistically significant differences ($P \leq 0.05$) in fish mortality. Comparisons were made between the challenged untreated group, the challenged treated groups, and the unchallenged group as well as between the low-dose and high-dose groups. For all analyses, the experimental unit was the tank because the treatment was administered to all fish in each tank. Cumulative mortality results were analyzed by logistic regression using the GENMOD procedure in SAS (SAS Institute 1999). The binomial distribution was assumed, and the logit link was used in the analyses. Body weight data were analyzed by an ANOVA appropriate for a repeated-measures experiment using the MIXED procedure in SAS. Histopathologic data were not analyzed statistically due to the finding of few abnormalities in unchallenged and florfenicol-medicated fish.

Results

Tolerance Study

Fish weight and feeding behavior.—The 10-d regimens of 0, 10, 20, 40, and 100 mg of florfenicol resulted in weight gains of 16.2, 15.2, 20.0, 14.0, and 14.5%, respectively. The palatability scores were 19.25, 19.50, 20, 19.25, and 19.5, respectively (out of a possible 20). No statistically significant differences in weight gain were observed between treatment groups. Palatability data were nearly identical for all treatment groups; these data were not statistically analyzed.

Pathology.—No morbidity or mortality was observed during the study. Only 6 of the 400 fish were found to have gross lesions during the postmortem examination. Mild mottling of the liver was seen in 3 of the fish from the 10-mg group and in 1 fish from the control group. Mild mottling of the spleen was seen in 2 fish from the 20-mg group and in 1 fish from the 10-mg group. There were no differences in the histopathology of the organs in untreated and treated fish.

Efficacy Study

Fish weight.—The mean fish weight at the time of challenge ranged from 47.9 to 66.5 g, whereas the mean fish weight at the end of the study (day 23) ranged from 40.3 to 81.2 g. After 23 d, untreated, challenged fish had significantly ($P \leq 0.05$) lower mean body weight than unchallenged or treated fish.

Feeding activity.—Feeding activity in the unchallenged, unmedicated fish was scored as 2 throughout the study. The feeding activity of the challenged fish that received florfenicol- medicated feed at 10, 20, or 40 mg of florfenicol per kilogram of body weight was also scored as 2 throughout the study. However, on day 5 the fish in two 10-mg tanks and one 40-mg tank had feeding scores of 1. The feeding activity of the majority of the challenged, unmedicated fish was observed to be either 1 or 0 on days 4–17. From day 18

TABLE 1.—Feeding behavior of channel catfish that were unchallenged or challenged with *Edwardsiella ictaluri* and given 0, 10, 20, or 40 mg of florfenicol per kilogram of body weight in feed for five consecutive days. Scores were assigned as follows: 0 = feeding poorly, 1 = ≤50% feed consumed, and 2 = 50–100% feed consumed. Values represent the means of four tanks on each day. Day 1 was the first day experimental diets were fed.

	Dose				
Day	0 (unchallenged)	0 (challenged)	10 (challenged)	20 (challenged)	40 (challenged)
0	2	2	2	2	2
1	2	2	2	2	2
2	2	2	2	2	2
3	2	2	2	2	2
4	2	1	2	2	2
5	2	1	1.5	2	1.75
6	2	1	2	2	2
7	2	1	2	2	2
8	2	1.25	2	2	2
9	2	0.75	2	2	2
10	2	0.75	2	2	2
11	2	0.25	2	2	2
12	2	0.25	2	2	2
13	2	0.25	2	2	2
14	2	0.25	2	2	2
15	2	0.25	2	2	2
16	2	0	2	2	2
17	2	0	2	2	2
18	2	1.5	2	2	2
19	2	1.5	2	2	2
20	2	1.5	2	2	2
21	2	2	2	2	2
22	2	2	2	2	2

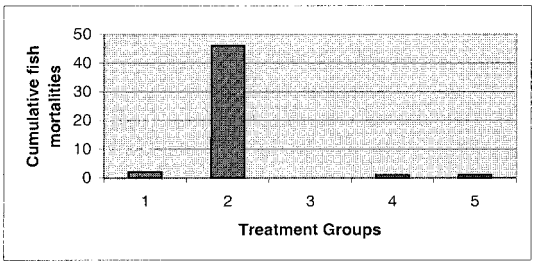

FIGURE 2.—Cumulative mortality of channel catfish that were unchallenged (treatment 1) or challenged with *Edwardsiella ictaluri* and given 0, 10, 20, or 40 mg of florfenicol per kilogram of body weight in feed for five consecutive days (treatments 2−5).

onward, the feeding response improved to either 1 or 2 in these fish (Table 1).

Mortality.—The first mortalities were identified in a challenged, nonmedicated control tank on day 7. Mortalities were seen in this group from day 7 to day 22, most of them occurring on days 8 (8.75% [7/80]), 9 (15% [12/80]), and 13 (7.5% [6/80]). In the challenged, nonmedicated control group, the total mortality rate was 57.5% (46/80), the individual tank mortality rates ranging from 35% (7/20) to 100% (20/20). The nonchallenged, nonmedicated control group had 2 mortalities (2.5% [2/80]). No mortalities occurred in the low-dose medicated group (10 mg/kg). The intermediate-dose (20-mg/kg) and high-dose (40-mg/kg) groups had one mortality each (1.25% [1/80]), on days 22 and 21, respectively. The cumulative mortality in each treated group and the nonchallenged control group (Figure 2) was significantly less than that of the challenged control group ($P < 0.0001$ for each contrast). There were no statistically significant pairwise contrasts among the florfenicol-treated groups and the nonchallenged control group.

Microbiology.—*Edwardsiella ictaluri* was recovered from 70% (56/80) of the fish in the challenged, unmedicated control group, compared with 10% (8/80) of those in the unchallenged, unmedicated control group. For the florfenicol-treated fish, *E. ictaluri* was recovered from 3.8% (3/80) of the fish in the 10-mg group, 2.5% (2/80) of those in the 20-mg group, and 5.0% (4/80) of those in the 40-mg group.

Bacterial colonies characteristic of *E. ictaluri* were cultured from 93.9% (46/49) of the dead fish recovered from 6 of the 20 tanks. Biochemical tests were performed on 25 of 46 isolates and confirmed them as *E. ictaluri* (McGinnis et al. 2003). Six tanks that did not yield mortalities (one of the unchallenged tanks, two of the 10-mg tanks, two of the 20-mg tanks, and one of the 40-mg tanks) had fish that cultured positive for *E. ictaluri*.

Pathology.—Gross examination of the fish revealed lesions consistent with ESC. External lesions included cranial midline ulcers (''holes in the head''), hemorrhages on the skin and fins, and exophthalmia. Internal lesions included gastrointestinal and liver hemorrhages, congested spleens, ascites, and swollen kidneys. These lesions were observed in 98.6% (72/73) of all cases (including cultures from mortalities and euthanatized fish) determined to be positive for *E. ictaluri*. The prevalence of these lesions was higher in the untreated, challenged group than in the treated and nonchallenged groups. There were no lesions indicative of any concurrent diseases.

Gross lesions indicative of ESC were observed in the challenged, untreated group ($n = 80$ fish) as follows: skin ulcers (43.8%), ascites (42.5%), gastrointestinal hemorrhage (26.2%), liver hemorrhage (18.8%), gill hemorrhage (18.8%), exop-

thalmia (16.2%), posterior kidney hemorrhage (15.0%), skin hemorrhage (15.0%), increased cranial midline pallor (10.0%), mouth hemorrhage (7.5%), mottled posterior kidneys (6.2%), and congested spleen (5.0%). Other lesions observed at frequencies less than 3.8% in unchallenged or florfenicol-medicated groups included congested posterior kidney, congested liver, posterior kidney hemorrhage, liver hemorrhage, mouth hemorrhage, skin hemorrhage, and increased cranial midline pallor.

Histopathologic examination showed an increased degree of inflammatory cell infiltrate in the liver, heart, gills, anterior kidney, and spleen of the untreated, challenged fish, compared with a paucity of such lesions in the unchallenged and the florfenicol-treated, challenged fish.

Discussion

In the efficacy study, treatment with medicated feed was initiated the day after inoculation and before the appearance of clinical signs. Because of this aggressive treatment, there were few mortalities in the florfenicol-medicated fish. Incorporating florfenicol into pelleted feed has been shown to be a highly effective method of administering it to fish (Samuelson et al. 1998). In commercial practice, it is recommended that feeding be initiated as soon as clinical signs are noted because anorexia is one of the clinical signs associated with ESC and this would prevent fish from consuming a therapeutic dose. In a field study with sarafloxacin, administration of medicated feed was not initiated until fish were microbiologically confirmed to be infected with *E. ictaluri*, which was 3 d after the first mortalities occurred (Johnson and Smith 1992). Many fish became infected and anorectic and died before receiving adequate levels of drug in the feed. The medicated fish nonetheless showed a statistically significant difference in survival compared with the unmedicated controls. Feeding at the first sign of disease will also help avoid bacterial resistance in channel catfish. A pathogen may be sensitive to the concentration of an antibiotic when fish are feeding well but not susceptible to the dose achieved in a fish with a loss of appetite.

The 57.5% mortality rate in untreated, challenged fish compared closely to previous reports. In a previous tank study of the efficacy of antibiotics in treating ESC, there were cumulative mortalities of 63% for a 4-h exposure and 69% for an 8-h exposure when fish were exposed to *E. ictaluri* at a concentration of 2.6×10^6 CFU per milliliter of water (Wise et al. 1997). In a field study with three separate trials, the mortality rates were 57.3, 88.3, and 41.46% (Johnson et al. 1992).

In this study, there were two mortalities and 10% *E. ictaluri* isolations in the unchallenged, unmedicated group. The dead fish were infected with *E. ictaluri*. There were also six other fish in this group from which *E. ictaluri* was isolated. The tank harboring them was probably contaminated by an adjacent infected tank.

In the United States, Romet is currently the only antibiotic approved for the treatment of ESC in channel catfish. In a study comparing different treatment regimes for ESC, it was found that using Romet as prescribed was not effective for the control of mortalities associated with *E. ictaluri* (Wise and Johnson 1998). Therefore, there is a need for an efficacious antibiotic to control mortality associated with ESC.

Decreased feed intake due to the poor palatability of Romet has been reported in channel catfish at dose rates of more than 50 mg per kilogram of body weight at a feeding rate of 1% of body weight. Florfenicol has been extensively administered to salmonids in feed in commercial operations for the treatment of systemic bacterial diseases in Norway, Chile, Japan, Canada, and the UK without any reduction in the consumption of medicated feed. In seawater trials, Atlantic salmon were fed florfenicol- medicated diets ranging from 1× to 10× the recommended dose (6.4–85.5 mg/kg) for 10 d without a reduction in the consumption of medicated feed. Fish were randomly selected for histological examination on the day after completion of the treatment as well as the day when the trials were terminated. A total of 100 fish were examined; no tissue abnormalities associated with the florfenicol treatment were observed, and fish in all groups gained weight. A similar study with Atlantic salmon in freshwater also revealed no abnormalities associated with florfenicol treatment (Inglis et al. 1991).

Concern about the resistance of human pathogens to antibiotics was the reason that the U.S. Food and Drug Administration instituted a restriction on the development of antibiotics for use in aquaculture. However, the usual mechanism of resistance in the two phenicol antibiotics used in human medicine (chloramphenicol and thiamphenicol) is not applicable to florfenicol. Bacteria that are resistant to chloramphenicol and thiamphenicol ordinarily produce a plasmid-mediated acetyltransferase (enzyme number 2.3.1.28; IUBMB 1992) that inactivates these antibiotics by acetylation of the 3-OH group.

Because florfenicol lacks this 3-OH group, it is unlikely that florfenicol will confer resistance to human pathogens (Shaw 1967; Fukui et al. 1987). In addition, when the concentration of phenicol and antimicrobials is less than 1/20th of the MIC value, these agents do not select for resistance. The most sensitive human enteric organism exhibited an MIC of 0.5 μg/mL with respect to florfenicol. The calculated enteric concentration of florfenicol-equivalent residues is 0.000244 μg/g, which is more than three orders of magnitude less than the MIC for 50% of the test organisms for the most sensitive organism in the human enteric flora (Dale Shuster, Schering-Plough Animal Health Corp., personal communication).

In the research reported in this article, 240 channel catfish were medicated with florfenicol in an efficacy study and 320 fish were medicated in a tolerance study. No adverse treatment- related effects were seen either grossly or histologically. There were no effects on feed consumption, demonstrating good palatability throughout the medicated feeding period, and all unchallenged and medicated groups gained comparable amounts of weight. Challenged, unmedicated fish generally lost weight, a reflection of their decreased feeding activity and the cachectic state induced by infection with *E. ictaluri*.

In conclusion, florfenicol administered for five consecutive days at daily dosages of 10, 20, and 40 mg/kg was efficacious in the control of experimentally induced *E. ictaluri* infections in channel catfish. These treatments increased survival and weight gain and did not cause pathological changes. This high order of efficacy indicates that dose confirmation and field efficacy trials are warranted to more accurately assess the potential for using florfenicol in commercial practice. Further, in the tolerance study, florfenicol administered for 10 consecutive days at daily doses of 10, 20, 40, and 100 mg/kg proved to be palatable to channel catfish fingerlings without causing specific lesions associated with the antibiotic.

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