Determination of Florfenicol Dose Rate in Feed for Control of Mortality in Nile Tilapia Infected with *Streptococcus iniae*

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**Abstract.**—A dose titration study was conducted to determine the dosage of florfenicol (FFC) in feed to control *Streptococcus iniae*-associated mortality in Nile tilapia *Oreochromis niloticus*. Six tanks were assigned to each of five treatments: (1) not challenged with *S. iniae* and fed unmedicated feed; (2) challenged with *S. iniae* by injection and fed unmedicated feed; (3) challenged with *S. iniae* and given FFC at 5 mg/kg of body weight (bw) in medicated feed; (4) challenged with *S. iniae* and given 10 mg FFC/kg bw; and (5) challenged with *S. iniae* and given 15 mg FFC/kg bw. Treatment was initiated the day after inoculation, and feed was administered for 10 d. Cumulative mortality was 0% in the unchallenged, untreated group; 35.8 ± 4.4% (mean ± SE) in the challenged, unmedicated group; 19.2 ± 2.7% in the 5-mg/kg treated group, 12.5 ± 3.8% in the 10-mg/kg group, and 2.5 ± 1.1% in the 15-mg/kg group. The cumulative mortality was significantly less in each challenged, FFC-treated group than in the challenged, unmedicated controls (5 mg/kg: *P* = 0.0156; 10 mg/kg: *P* = 0.0007; 15 mg/kg: *P* < 0.0001). The efficacy of the 10- and 15-mg/kg FFC dosages was studied in a separate dose confirmation study. Fish in all tanks were injected with *S. iniae*. At 4 h postinoculation, 10 tanks were assigned to each of three feed treatments: (1) unmedicated feed; (2) 10 mg FFC/kg bw; and (3) 15 mg FFC/kg bw. Cumulative mortality was 20.5 ± 2.0% in the challenged, unmedicated group; 11.0 ± 2.1% in the 10-mg/kg group, and 5.5 ± 2.4% in the 15-mg/kg group. Mortality was significantly less in the medicated groups than in the challenged, unmedicated controls (10 mg/kg: *P* = 0.0270; 15 mg/kg: *P* = 0.0007). Fish in both studies were necropsied, cultured for bacteria, and examined for gross lesions. The minimum inhibitory concentration of FFC against *S. iniae* in both studies ranged from 0.5 to 1.0 μg/mL. Florfenicol was palatable, safe, and efficacious for control of Nile tilapia mortality due to *S. iniae* infection.

*Streptococcus iniae* infection is considered to be the most important bacterial disease of cultured tilapias *Oreochromis* spp., causing mass mortality and severe economic losses (Shoemaker et al. 2001). Currently, there are no approved antibiotics or vaccines in the USA for control of this disease. Under the Animal Medicinal Drug Use Clarification Act, veterinarians can prescribe sulfa-dimethoxine/ormetoprim (Romet) and oxytetracycline (OTC; USDA CVM 2008), but a lack of efficacy and resistance of bacteria to these antibiotics have been reported (Stoffregen et al. 1996; Shoemaker and Klesius 1997). In addition, there are palatability problems with medicated feeds administered to fish containing sulfa-dimethoxine/ormetoprim combinations (Plumb 1999). Until recently, OTC-medicated feed was manufactured only as a sinking pellet, making it difficult to gauge feeding activity of infected fish. Although OTC can be incorporated into a floating feed, availability of this product is limited.

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Received October 16, 2009; accepted April 30, 2010

Published online July 28, 2010
Florfenicol (FFC) is widely available as a medicated feed and is highly efficacious against many fish pathogens (Fukui et al. 1987; Inglis and Richards 1991; Samuelson et al. 1998; Gaunt et al. 2003). In 2005, FFC was approved for catfish (USFDA CVM 2009) and salmonids (USFDA 2007) in the USA. We report the results of two studies (dose titration and dose confirmation) to determine an efficacious FFC dose rate for control of tilapia mortality associated with *S. iniae*.

**Methods**

*Fish.*—Six-hundred commercially reared, genetically male fingerlings of Nile tilapia *Oreochromis niloticus* (Til-Tech Aquafarm, Robert, Louisiana) with no known history of exposure to *S. iniae* were used for each study. The fish in the dose titration study were 8 weeks old, and those in the dose confirmation study were 12 weeks old. These fish were held in separate 1,200-L vats in the Wet Laboratory at the Thad Cochran National Warmwater Aquaculture Center (Stoneville, Mississippi) until acclimation. All fish were considered naïve because they had never been exposed to a *S. iniae* epizootic. Serum antibody titers to *S. iniae* were determined by a modified agglutination assay (Conrath 1972) in 25 arbitrarily selected nonexperimental fish. Results were negative for all fish. Throughout all phases of these studies, fish were monitored for possible adverse events, such as low dissolved oxygen or unexpected mortality. No adverse events were noted in either study.

*Feeding behavior observations.*—Feeding activity was assessed as follows: fish were hand fed once per day, and feeding activity was visually observed and recorded throughout the study. A numerical score was assigned to each tank based on the approximate percentage of feed consumed (3 = 75–100%; 2 = 50–75%; 1 = 25–50%). A score of 0 was assigned if no feed consumption was detected.

*Environmental parameters.*—Thirty glass tanks (80 L) supplied with aquifer well water (single-pass flow-through) were used for each study. Water supply to the tanks was analyzed for lead, copper, organochlorines (OCl), and organophosphorus compounds (OP) prior to the studies. Water quality attributes were measured once weekly during each study; measured variables included temperature, pH, total ammonia, chloride, and nitrite. Alkalinity and hardness were measured once prior to each study. All results of water quality assays during these studies were within acceptable limits for maintenance of Nile tilapia fingerlings. No harmful levels of OP, OCl, or metals were detected. The photoperiod was set to 12 h light : 12 h dark.

*Experimental design of the dose titration study.*—Six-hundred fish were arbitrarily assigned equally to 30 tanks (20 fish/tank) during the dose titration study. Six tanks were randomly assigned to each of five treatment groups: (1) not challenged with *S. iniae* and fed unmedicated feed (negative control); (2) challenged with *S. iniae* and fed unmedicated feed (positive control); (3) challenged with *S. iniae* and given feed medicated with 5 mg of FFC/kg of body weight (bw); (4) challenged with *S. iniae* and given 10 mg FFC/kg bw in medicated feed; and (5) challenged with *S. iniae* and given 15 mg FFC/kg bw in medicated feed.

Fish were acclimated to experimental conditions for 8 d during which they were fed a commercial tilapia diet once per day at an estimated rate of 2.5% bw. On day 0 after acclimation, the 20 fish from each tank were weighed as a group to calculate the mean individual weight. The fish weights ranged from 27.1 to 34.8 g. Each fish received an intracoelomic (IC) injection with 0.1 mL of inoculum containing *S. iniae* at approximately 10⁵ colony-forming units (CFU)/mL or broth without bacteria (control); feed treatments were initiated the day after inoculation. Fish were given their treatment feed once per day at 2.5% bw for 10 consecutive days (days 1–10), and their feed responses were recorded. Water temperature (mean ± SE) was 21.4 ± 0.08°C during the study. Mortalities were recorded and removed from tanks twice daily, and the quantity of feed administered to those tanks decreased proportionally to the reduced tank biomass based on mean fish weight. All dead fish were necropsied and cultured for bacterial isolation. After the 10-d treatment period, fish were observed twice daily for 17 d while receiving a commercial (unmedicated) tilapia feed (Burris Mill & Feed, Franklinton, Louisiana) at a rate of 2.5% bw. At the end of the observation period, surviving fish were removed from each aquarium, counted, and weighed as a group. The fish were then euthanized and submitted for microbiological and pathological evaluation.

*Experimental design of the dose confirmation study.*—Six-hundred fish were arbitrarily assigned equally to 30 tanks in the dose confirmation study, and 10 tanks were assigned to each of three treatment groups: (1) challenged with *S. iniae* and fed unmedicated feed; (2) challenged with *S. iniae* and given medicated feed at 10 mg FFC/kg bw; and (3) challenged with *S. iniae* and given medicated feed at 15 mg FFC/kg bw. Fish were first acclimated to experimental conditions for 6 d and then were weighed and challenged on day 0. Weight ranged from 4.5 to 9.5 g. Fish were inoculated with *S. iniae* in a similar manner and were fed the same dose for 10 d. At the end of the treatment period, fish were weighed and challenged.
fashion as described for the dose titration study. Treatments began 4 h after IC injection with *S. iniae*. Previously, we conducted another dose confirmation study (authors’ unpublished data) in which the fish were infected on day 0 and treatment began on day 1. By feeding time on day 1, the infected fish were lethargic and anorexic; their feed intake was less than 25% in all groups. We completed the study, analyzed mortality and infection rate data, and observed no difference between unmedicated and FFC-mediated groups. Cumulative mortality rates in the 0-, 10-, and 15-mg/kg treated groups were 30.5, 27.5, and 32.0%, respectively. Therefore, to ensure that fish consumed the medicated diets, feeding of assigned treatments began at 4 h postchallenge and continued once daily for a total of 10 consecutive days. The mean water temperature was 27.2 ± 0.08°C during the study. Fish were fed and monitored during the treatment phase and for a 15-d observation period after treatment as described above. At the end of the study, all surviving fish were fed and monitored during the treatment phase and for a 15-d observation period after treatment as described above.

Preparation of feeds.—Commercial tilapia feed containing 35% crude protein (Burris Mill & Feed) was used for the dose titration and dose confirmation studies. The feed was ground and repelleted as previously described (Li et al. 1993). For preparation of medicated feeds, FFC 50% premix (Aquaflor 50% type A medicated article; Intervet/Schering Plough Animal Health, Roseland, New Jersey) was mixed with the dry ground feed and then pelleted. For the dose titration study, feed containing FFC at 0, 200, 400, and 600 mg/kg of feed was prepared in order of increasing FFC concentration. For the dose confirmation study, feed containing 0, 400, and 600 mg FFC/kg of feed was also prepared in increasing FFC dose concentrations. Feeds were stored in a monitored refrigerator at 4 ± 2°C prior to use.

Analysis of medicated feed.—Eurofins Scientific, Inc. (Memphis, Tennessee), analyzed all feeds for FFC content (Hayes 2005). The concentration of FFC in feed for the dose titration study was 195 mg/kg (97.5% of nominal) for the 200-mg/kg feed, 390 mg/kg (97.5% of nominal) for the 400-mg/kg feed, and 612 mg/kg (102% of nominal) for the 600-mg/kg feed. In the dose confirmation study, the concentration of FFC in the medicated feed was 426.8 mg/kg (106.7% of nominal) for the 400-mg/kg feed and 587.5 mg/kg (97.9% of nominal) for the 600-mg/kg feed. The FFC concentrations in the nonmedicated feed were all below the limit of quantitation. Eurofins Scientific also analyzed all feed for OTC and sulnadimethoxine/ormetoprim residues, and SGS Agricultural Services (Memphis, Tennessee) analyzed all feeds for OP and OCI residues. None of these potential contaminants were found at concentrations that would adversely affect the outcome of the study.

Preparation of Streptococcus iniae inoculum and challenge of fish.—The *S. iniae* isolate (L00-300) was provided by John Hawke (Fish Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University, Baton Rouge). The isolate was identified by biochemical characteristics (Perera et al. 1994; Agnew and Barnes 2007) using a bacterial identification system according to manufacturer’s instructions (Difco; Becton, Dickinson and Company, Sparks, Maryland; API 20 Strep Identification System; bio-Merieux, Inc., Durham, North Carolina). Using Sensititre plates (TREK Diagnostic Systems, Inc., Cleveland, Ohio), the minimal inhibitory concentration (MIC) of FFC for the *S. iniae* inoculum isolate was identified as 1 μg/mL.

The *S. iniae* isolate was cultured in Todd–Hewitt broth (Difco Laboratories, Detroit, Michigan) at 28 ± 2°C for 24 h. To optimize pathogenicity prior to the dose titration and confirmation studies, 0.1 mL of *S. iniae* in Todd–Hewitt broth was administered by IC injection into each of four passage fish. These inoculated fish died at 1–4 d postinoculation and were submitted for gross examination and bacterial culture. Brains and spleens from these inoculated fish were cultured on Mueller–Hinton agar with 5% sheep blood at 25 ± 1°C. To produce the inoculum, two to three recovered colonies were placed into each of four test tubes with Todd–Hewitt broth for the dose titration study and into five tubes for the dose confirmation study.

The reisolated *S. iniae* was passed two more times (as described above) for a total of three passages to ensure virulence. A sterile swab was used to inoculate the *S. iniae* into 10 mL of Todd–Hewitt broth, and the *S. iniae* culture was incubated at 28 ± 2°C overnight prior to challenge. After incubation, the overnight culture containing the *S. iniae* was decanted into 250 mL of Todd–Hewitt broth and was placed in an incubator with a stir bar for agitation; 5 mL of inoculated broth were then removed from each liter and serially diluted to perform colony counts of bacteria as well as purity checks.

Prestudy trials indicated that for the *S. iniae* isolate used in this study, the dose that was lethal to 50% of test fish (LD50) was 10^5 CFU/mL (authors’ unpublished data), which is in agreement with previously reported *S. iniae* LD50s (Bromage et al. 1999; Eldar and Ghittino 1999). Six tubes of the 10^5-CFU/mL concentration were prepared for inoculation of the Nile tilapia fingerlings. Each fingerling was injected with
0.1 mL of bacteria in Todd–Hewitt broth. Fish from control tanks were injected with broth only.

Microbiological methods.—In the dose titration and dose confirmation studies, cultures for bacterial pathogens were collected from the brain and spleen of each fish and were streaked onto Mueller–Hinton agar plates (Becton, Dickinson and Company) containing 5% sheep blood. Streptococcus iniae was identified on the basis of biochemical results through commercially available testing kits as described previously. Quality control for the bacterial identification system was performed in accordance with manufacturer's recommendations using Streptococcus equi spp. zooepidemicus (ATCC [American Type Culture Collection] 700400).

Disk diffusion zones of inhibition were performed for all identified S. iniae in each study to assess the susceptibility to FFC. Colonies were suspended in a suspension medium (bioMerieux) using a sterile swab and standardized to a 0.5 McFarland turbidity standard. The swab was then used to uniformly streak the bacteria onto a Mueller–Hinton agar plate (Becton, Dickinson and Company) containing 5% sheep blood. A FFC-impregnated disk (Difco Laboratories) was then placed in the middle of the plate. Inoculated plates were incubated at 28 ± 2°C until the zone of inhibition was clearly defined. Zones were measured in millimeters using a certified caliper.

The MIC was determined for the inoculum bacteria prior to inoculation and for selected cultures during each study. A tube of sterile distilled water was placed in the middle of the plate. Inoculated plates were incubated at 28 ± 2°C for 18–24 h. Results were read using a light viewer to display the underside of the wells. Growth appeared as turbidity or a deposit of cells (button) on the bottom of the well. The MIC was recorded as the lowest concentration of antimicrobial that inhibited visual growth. The MIC for the inoculum bacteria was 1 μg/mL in both studies.

Gross necropsy.—Dead fish were removed and submitted for necropsy. Morbid fish were euthanized with an overdose of tricaine methanesulfonate (Tricaine-S; Western Chemical, Inc., Ferndale, Washington) prior to necropsy. Postmortem examination of fish included gross inspection for lesions of the skin, fins, vent, eyes, and coelomic viscera and microscopic examination for parasites of the gill filaments.

Statistical analysis.—Data describing fish mortality and tissue culture for S. iniae were evaluated for statistically significant differences (P < 0.05). In the dose titration study, comparisons were made (1) between the challenged, untreated group and each of the other groups (the unchallenged, untreated group and the three challenged, FFC-treated groups) and (2) between the low-dose (5 mg/kg) and high-dose (15 mg/kg) FFC-treated groups. In the dose confirmation study, comparisons were made between the untreated group and the treated groups. Cumulative mortality was analyzed using the GLIMMIX macro in the Statistical Analysis System (SAS) version 8.02 (SAS Institute 1999). Logistic distribution, logit link, and the Kenward–Rogers approximation for degrees of freedom were used to analyze cumulative mortality data, with treatment as the fixed effect and tank as the random effect.

To test the strict inequality that treatment with FFC medicated feed was more efficacious than nonmedicated feed, two-tailed t-tests were calculated prior to challenge and administration of medicated feed and one-tailed tests were run after treatment. The linear–plateau analyses used one-tailed tests. Statistical analysis was performed using SAS.

Mean fish weights were calculated by weighing the 20 fish/tank in a tared bucket of water on the day of challenge. The number of fish in the tank was factored to yield mean fish weight per tank. An analysis of variance (ANOVA) was performed to demonstrate that there was no prechallenge difference in average allocated fish weight. The surviving fish from each tank were weighed at the conclusion of the study, and those results were analyzed by ANOVA using weight on day 0 as a covariate.

Results

Dose Titration Study

After inoculation of the fish on day 0, the first mortalities were identified in a challenged, unmedicated control tank on day 2. Mortalities were seen in this group from day 2 to day 20, with the majority occurring on days 2 (10.0%, 12 of 120 fish), 3 (12.5%, 15 of 120), and 4 (5.1%, 6 of 120). The mean (±SE) cumulative percent mortality (Figure 1) of fish in this group was 35.8 ± 4.3%, with the cumulative
tank mortalities ranging from 20% (4 of 20 fish) to 45% (9 of 20). The unchallenged, unmedicated control group had 0% mortality (0 of 120 fish). The low-dose (5 mg/kg) FFC-medicated group had mortalities from day 3 to day 11, with the majority occurring on days 4 (5.8%, 7 of 120 fish), 5 (5.0%, 6 of 120), and 6 (4.2%, 5 of 120) for a cumulative mortality rate of 19.2 ± 2.7% (23 of 120). The majority of mortalities in the middle-dose (10 mg/kg) medicated group occurred on days 5 and 6 (3.3%, or 4 of 120 fish, for each occurrence) for a cumulative mortality rate of 12.5 ± 3.8% (15 of 120). The high-dose (15 mg/kg) medicated group had one mortality on each of three days (days 2, 5, and 6; 0.83%, or 1 of 120 fish, for each occurrence) for a cumulative mortality rate of 2.5 ± 1.1% (3 of 120; Figure 1). The mortality in each challenged, FFC-treated group and the unchallenged control group was significantly less than that of the challenged, unmedicated control group (5 mg/kg: $P = 0.0156$; 10 mg/kg: $P = 0.0007$; 15 mg/kg: $P < 0.0001$).

In the challenged, unmedicated control group, *S. iniae* was cultured from 35% of the fish (42 of 120). In the unchallenged, unmedicated control group, *S. iniae* was not cultured from any of the fish. *Streptococcus iniae* was recovered from 19.2% of fish (23 of 120) in the 5-mg/kg treated group, 12.5% of fish (15 of 120) in the 10-mg/kg treated group, and 2.5% of fish (3 of 120) in the 15-mg/kg treated group. *Streptococcus iniae* was cultured from 98.7% of dead fish (82 of 83) recovered from 26 of the 30 tanks. The pathogen was not cultured from any of the surviving fish or from fish in the four challenged, FFC-treated tanks that did not yield mortalities.

Postmortem examination of mortalities revealed lesions compatible with *S. iniae* infection (Perera et al. 1994; Al-Harbi 1996; Plumb 1999; Colomí et al. 2002; Darwish 2007; Bromage and Owens 2009). External lesions included exophthalmia and hemorrhages in the fins, skin, and vent. Internal lesions included ascites, congested spleen, and gastrointestinal and hepatic hemorrhages. Gross lesions typical of *S. iniae* infection were observed in 67.8% of fish (57 of 84) that tested positive for *S. iniae*. The prevalence of these lesions was higher in the untreated group than in the FFC-treated groups. No lesions indicative of any concurrent disease and no specific gross lesions that could be associated with *S. iniae* were observed in this study. The bacteria recovered from dead fish had disk diffusion zones that ranged from 24 to 29 mm and MIC assay results that ranged from 0.5 to 1.0 μg/mL.

Mean fish weight at the time of challenge ranged from 27.1 to 34.8 g, and the mean weight of survivors at the end of the study ranged from 39.8 to 53.6 g. There was no significant difference noted in the feeding activity between the challenged, untreated fish and the challenged, treated fish. The mean feeding activity score during the treatment period was 2.90 for the unchallenged, untreated fish and 2.60 for the challenged, untreated fish. The mean feeding activity score of FFC-medicated fish ranged from 2.75 to 2.80 during treatment. The least feeding activity in the medicated groups was seen on day 1, when 6 of the 18 FFC-medicated tanks had a feeding activity score of 1; 8 of the 18 tanks had a feeding activity score of 2; and 4 of the 18 tanks had a feeding activity score of 3. From day 3 onward, all FFC-medicated tanks had feeding scores of either 2 or 3. From day 13 onward, the feeding response in both treated and untreated tanks improved to a score of 3.

**Dose Confirmation Study**

After inoculation of the fish on day 0, the first mortalities were identified in a challenged, unmedicated control tank on day 2. Mortalities were seen in this group from day 2 to day 16, with the majority occurring on days 2 (8.0%, 16 of 200 fish), 3 (7.5%, 15 of 200), and 4 (2.0%, 4 of 200). The mean (±SE) cumulative percent mortality (Figure 2) in the challenged, unmedicated control group was 20.5 ± 2.0%,
with the cumulative tank mortalities ranging from 15% (3 of 20 fish) to 35% (7 of 20). In the 10-mg/kg FFC-treated group, most mortalities occurred on days 3 (4.5%, 9 of 200 fish), 4 (2.0%, 4 of 200), and 6 (1.5%, 3 of 200) for a cumulative mortality rate of 11.0 ± 2.1% (22 of 200). The 15-mg/kg treated group had the majority of mortalities on days 3 (2.0%, 4 of 200 fish) and 4 (1.5%, 3 of 200) for a cumulative mortality rate of 5.5 ± 2.4% (11 of 200; Figure 2). The mortality rate for the challenged, unmedicated group was significantly higher than that of the two FFC-medicated groups (10 mg/kg; $P = 0.0270$; 15 mg/kg; $P = 0.0007$). The two medicated groups were not significantly different ($P = 0.0855$).

Streptococcus iniae was recovered from 19.5% of the fish (39 of 200) in the challenged, unmedicated control group; 10% of the fish (20 of 200) in the 10-mg/kg FFC-treated group; and 4.5% of the fish (9 of 200) in the 15-mg/kg treated group. Streptococcus iniae was cultured from 89.2% of dead fish (66 of 74) recovered from 23 of 30 tanks.

Streptococcus iniae was recovered from one fish in the challenged control group and from one fish in a 10-mg/kg FFC-treated tank that survived challenge. The five tanks that did not yield mortalities (all FFC treated) had no S. iniae-positive cultures. Streptococcus iniae was not recovered from surviving fish that received FFC at 15 mg/kg. Gross lesions as described in the dose titration study were seen in 52.9% of fish (36 of 68) identified as being S. iniae positive. The prevalence of these lesions was increased in the untreated group compared with the treated groups. No lesions indicative of any concurrent disease and no specific gross lesions that could be associated with FFC were observed in this study. The disk diffusion zones ranged from 24 to 26 mm, and the MIC ranged from 0.5 to 1.0 μg/mL.

Mean fish weight at the time of challenge ranged from 4.5 to 9.5 g, and the mean weight of survivors at the end of the study (day 24) ranged from 12 to 13 g. There was no significant difference noted in the feeding activity between the challenged, untreated fish and the treated fish; the mean feeding activity score of all treatment groups was 3.0 from acclimation to the termination of the study.

**Discussion**

Initiation of FFC treatment within 24 h after exposure to S. iniae in the dose titration and dose confirmation studies produced significantly higher survival rates compared with the challenged, unmedicated group. The cumulative mortality in the dose titration study ranged from 2.5% to 19.2% in FFC-treated fish compared with 35.8% in the challenged, unmedicated group. In the dose confirmation study, mortality ranged from 5.5% to 11.0% in FFC-treated fish compared with 20.5% in the challenged, unmedicated group. Differences in cumulative mortality between the 10- and 15-mg/kg FFC-treated groups were significant only in the titration study. Because the approved FFC dose rate reported for catfish and salmonids is 10 mg/kg daily for 10 d (USFDA 2007, 2009), we included the 10-mg/kg dose rate in the dose confirmation study to verify whether there was an efficacy difference between the 10- and 15-mg/kg dose rates in Nile tilapia. Although the difference in cumulative mortality rates between the two FFC-treated groups was not significant in the dose confirmation study, the cumulative mortality percentage was lowered from 11.0% in the 10-mg/kg group to 5.5% in the 15-mg/kg group. The finding of the lower (but not significantly lower) mortality rate at 15 mg/kg than at 10 mg/kg is in agreement with reports of FFC’s efficacy against S. iniae in sunshine bass (female white bass Morone chrysops × male striped bass Morone saxatilis; Darwish 2007). One surviving fish from the 10-mg/kg group in the dose confirmation study cultured positive for S. iniae at termination. No survivors in the 15-mg/kg group were culture positive.
reservoir for future infections (Agnew and Barnes 2007). Because of the complete lack of carriers and the increased efficacy, a dose rate of 15 mg/kg should be considered in the treatment of tilapia infected with S. iniae. Farmers potentially using these medicated feeds would also need to consider cost differences between these FFC-mediated feed dosages in their selection of a dose rate. Regardless of which dose rate is used, farmers should closely monitor fish health and begin immediate treatment if warranted because of the rapidity with which fish began dying in these studies.

Several techniques have been reported for S. iniae challenge, including IC inoculation, immersion with abrasion, and cohabitation (Al-Harbi 1996; Chang and Plumb 1996; Bowser et al. 1998; Plumb 1999; Evans et al. 2001; Ndong et al. 2007). Based on a review of these aforementioned studies and personal communication with scientists researching Streptococcus disease in tilapias, IC injection was chosen as the most expeditious method to induce infection. Different species of tilapia are used in challenge models, including blue tilapia O. aureus), Nile tilapia, and Mozambique tilapia O. mossambicus (Al-Harbi 1996; Chang and Plumb 1996; Bowser et al. 1998; Darwish et al. 2002). Although the Nile tilapia is reported to be the most S. iniae resistant among the tilapia species, we chose this species for our studies and personal communication is the most popularly cultured tilapia outside of Africa (Popma and Masser 1999).

The mortality percentages from our untreated, IC-challenged Nile tilapia were comparable with other reports for the same species. Previous reports include a 30% mortality rate after IC injection (Al-Harbi 1996) and a 40% mortality rate after immersion challenge (Chang and Plumb 1996; Shoemaker et al. 2000). Variables such as stocking density, temperature, stress, tilapia strain, dose rate, and length of immersion exposure are reported to influence mortality rates (Perera et al. 1997; Le Morvan et al. 1998; Plumb 1999; Ndong et al. 2007; Zhou et al. 2008; Bromage and Owens 2009). Although we attempted to obtain uniform Nile tilapia for both studies, the two groups of naïve juveniles shipped from the supplier differed in age and size. As a result of the dissimilarities, the fish density was greater in our dose titration study (7.7 g of fish/L) than in the dose confirmation study (1.9 g of fish/L). However, this was unlikely to have caused the differences in mortality rates of the untreated control fish in the dose titration study versus the dose confirmation study because loading densities only in excess of 11.2 g of fish/L were shown to be an important risk factor for mortality of S. iniae-infected Nile tilapia (Shoemaker et al. 2000). Streptococcus iniae is reported to infect adult, subadult, and juvenile tilapia (Agnew and Barnes 2007). Further, there are no published reports indicating that infection rates differ between 8- and 12-week-old juvenile tilapia.

We also considered that fish size could affect the FFC plasma and tissue concentrations in medicated fish (and hence create a difference in efficacy of the antibiotic). However, in a study assessing the effect of Nile tilapia size (range = 100–500 g) on FFC concentration in plasma and muscle, the authors concluded that the dose rate of 15 mg/kg administered once daily for 10 d achieved a plasma or tissue concentration in each group that exceeded the MIC for S. iniae (Bowser et al. 2009). Because of sampling difficulty, there are no reported FFC kinetic studies using juvenile fish. Bowser et al. (2009) demonstrated that size differences in subadult and adult Nile tilapia did not affect the plasma or tissue distribution of FFC required for its antimicrobial activity. Based on the feeding activity and significantly decreased mortality of juveniles in the FFC-mediated groups in our studies, we do not believe that there were significantly different FFC concentrations in the fish plasma and tissues in either the dose titration study or the dose confirmation study. Regardless, the primary factor reported to cause differences in antimicrobial responses is the pharmacodynamic variation of different strains of an organism (AliAbadi and Lees 2000). However, the inoculum for both studies was the same S. iniae isolate, and the MIC value ranged from 0.5 to 1.0 μg/mL for all bacteria cultured throughout both studies. These data in combination with the findings of the Nile tilapia kinetic study by Bowser et al. (2009) indicated that an efficacious FFC concentration against the S. iniae isolate was achieved in each of the studies.

The difference in the mortality rates between the dose titration and dose confirmation studies may reflect natural variability in fish, differences in mean water temperature (21.4°C versus 27.2°C), or both factors. Tilapia are cultured at temperatures between 20°C and 35°C, with an optimum range of 27.7–30.0°C (Rakocy and McGinty 1989). Water temperatures in other experimental studies conducted with S. iniae-infected fish ranged from 21°C to 30°C (Eldar et al. 1995; Al-Harbi 1996; Chang and Plumb 1996; Perera et al. 1997; Bowser et al. 1998; Bromage et al. 1999; Evans et al. 2001; Darwish et al. 2002; Bromage and Owens 2009). Although reported S. iniae challenges differ in their temperature optima, our findings indicate that a challenge conducted at a mean water temperature of 21°C produced higher mortalities in controls than a challenge conducted at 27°C. This is in agreement with previous reports (Perera et al. 1997; Darwish et al. 2002). In a study comparing the effect of temperature in streptococcal disease manifestation, significantly
higher mortality rates were seen in experimentally infected Nile tilapia × blue tilapia hybrids maintained at 20°C than in fish maintained at 15, 25, 30, or 35°C (Perera et al. 1997). Mozambique tilapia that were challenged in 19, 23, 27, 31, or 35°C water exhibited decreased immune functions together with decreased resistance to \( S. \text{iniae} \) at 19°C or 35°C (Ndong et al. 2007).

Disk diffusion and MIC breakpoints for aquatic pathogens are not formally established by the Clinical and Laboratory Standards Institute (Miller and Reimischuesel 2006). However, disk diffusion zones of inhibition and MIC values from our studies are in agreement with FFC values reported for other aquatic pathogenic bacteria (Fukui et al. 1987; Smith 2006).

In conclusion, the data from these studies demonstrate that FFC administered to \( S. \text{iniae} \)-infected Nile tilapia for 10 d at a dose rate of either 10 or 15 mg/kg can significantly reduce mortality.

References


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