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Safety of Aquaflor (Florfenicol, 50% Type A Medicated Article), Administered in Feed to Channel Catfish, *Ictalurus punctatus*

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ABSTRACT

Aquaflor, a feed premix containing the broad spectrum antibacterial agent florfenicol (50% w/w), is being developed for use to control enteric septicemia (ESC) in channel catfish *Ictalurus punctatus* caused by the gram-negative enterobacterium *Edwardsiella ictaluri*. The recommended dose of Aquaflor to control ESC is 10 mg/kg body weight (BW)/day for 10 days. The study objective was to determine the safety of Aquaflor administered in feed to channel catfish at doses of 0 (control), 10, 30, and 50 mg/kg BW/day for 20 consecutive days. Parameters evaluated included daily mortality, behavioral (appetite, distribution, flight/fright response), and water chemistry observations, initial and terminal weight measurements, and gross and microscopic pathology. Medicated feed consumption was 67–86% of target with group mean doses of 8.5 mg/kg BW/day, 24.6 mg/kg BW/day, and 34.9 mg/kg BW/day. There were no mortalities or clinically observable changes noted at any of the dose levels tested. Aquaflor-related changes were limited to the food consumption and histopathology data. Although Aquaflor-related decreased feed consumption was noted in the 30 and 50 mg/kg BW/day groups, there were no differences in fish growth among the treatment groups. Aquaflor-related histopathology findings were limited to a histomorphologically evident dose-dependent decrease in hematopoietic/lymphopoietic tissue in the anterior kidneys, posterior kidneys, and spleens of channel catfish.

Keywords. Aquaflor; florfenicol; channel catfish; enteric septicemia; medicated feed.

INTRODUCTION

Aquaflor, a feed premix containing the broad spectrum antibacterial agent florfenicol at a concentration of 50% (w/w), is presently registered for use in Japan, South Korea, Norway, Chile, Canada, and the United Kingdom for the treatment of susceptible bacterial diseases in several fish species. Florfenicol is a fluorinated analog of thiamphenicol (10) specifically developed for use in veterinary medicine as an alternative to chloramphenicol, an antibiotic banned for use in food-producing animals (35). The primary use among salmonid species is for the treatment and control of furunculosis caused by *Aeromonas salmonicida*.

Channel catfish production represents about two-three of US freshwater aquaculture. Enteric septicemia (ESC) in channel catfish caused by the gram-negative enterobacterium *Edwardsiella ictaluri* causes serious production losses in cultured channel catfish. The causative bacterium is relatively host-specific for channel catfish; however, isolates have been recovered from various other ictalurid species (32). Although experimental infections have been established in blue tilapia *Oreochromis aureus*, chinook salmon *Oncorhynchus tshawytscha* and rainbow trout *Oncorhynchus mykiss*, natural infections in these fishes have not been described (4, 25). The disease is highly seasonal, with epizootics most common and mortalities greatest when water temperatures are between 22.2 and 27.8°C (32). Within this temperature range, fish rapidly reduce feeding and large numbers of sick or dead fish are observed with little if any initial period of low-grade losses. Mortality associated with ESC is generally lessened at marginal water temperatures of 18.3 to 22.2°C or 27.8 to 29.4°C, however infected fish are prone to secondary disease outbreaks. Mortality is rare when the water temperature is consistently below 18.3°C or above 29.4°C. Aquaflor is currently being developed to control ESC in channel catfish. Although data are available to support the safe use of Aquaflor in salmonids (17), similar data do not exist for channel catfish *Ictalurus punctatus*. The study objective was to determine the safety of florfenicol, Aquaflor (50% Medicated Type A Article), administered in feed to channel catfish for 20 consecutive days (2× the recommended treatment duration of 10 consecutive days) at 1×, 3×, and 5× the recommended dose rate of 10 mg/kg body weight (BW)/day. Because ESC epizootics are highly temperature-dependent, and drug metabolism is often temperature-dependent in poikilotherms, we chose to conduct this study at a temperature near the upper boundary of the ESC epizootic range.

MATERIALS AND METHODS

Test Fish

Channel catfish eggs were obtained from the Senecaville State Fish Hatchery (Ohio Department of Natural Resources, Senecaville, Ohio, USA), hatched and fish maintained at the Upper Midwest Environmental Sciences Center until transfer to the experimental tanks (∼10 months). The culture water temperature was increased from ∼12°C to 27°C at about 1°C per day over a 17-day period, after which the temperature was maintained at 27 ± 2°C for 6 weeks before transfer to the experimental tanks. Fish were held under an approximately 12-hour light: 12-hour dark photoperiod regime using incandescent lighting throughout the study. Fish were offered feed

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at ~2% BW/day. Fish were initially offered a commercial trout diet (Nelson and Sons Inc, Murray, Utah, USA) before conversion to the control diet ~2 months before dosing. Feed was withheld from fish on the day that they were transferred to the experimental tanks. All fish were used regardless of gender, and no attempt was made to determine gender or sexual maturity.

Study Design

The study consisted of 4 treatment groups (nominal doses of 0, 10, 30, and 50 mg/kg BW/day) each in 3 replicates with 20 channel catfish per replicate. Twelve fiberglass tanks (~0.55 m diameter, ~0.46 m depth, ~80 L volume) were arranged in 3 blocks with 4 tanks per block. Tanks were randomly allocated to treatment group according to a randomized block design, with 1 experimental tank per treatment group per block. After a 14-day acclimation period, each treatment group was offered one of the experimental diets for a period of 20 consecutive days (days 0–19) at about 2% BW to ensure complete consumption of experimental diets. Technicians offering the feed were unaware of the treatment assigned to each tank. Feed was offered once daily at least 1 hour after tank cleaning, except that fish were not fed until 24 hours after being allocated to the experimental tanks and fish were not fed after the last dose day. The amount of feed offered was initially based on the biomass in the experimental tanks at allocation and then on an estimated daily growth rate formula for channel catfish at UMESC (MP Gaikowski, unpublished data):

$$W_i = I_j + D \times 0.3018$$

where W_i is the weight at the end of period i, I_j is the average fish weight at the end of period j (the previous period), D is the number of days over which growth is estimated (5 or 7), and 0.3018 is the slope (g/d). The amount of feed to be offered was adjusted every seventh (acclimation) or fifth (dosing) day so that feed was offered at ~2% BW per day during the next 7-(acclimation) or 5-(dosing) day period.

Fiberglass fish tanks fitted with a translucent plexiglass lid with a feeding hatch were used in the study. Each block of 4 tanks received unchlorinated well water from an individual head box supplied with water at 27°C from a computer-controlled valve. Each tank within a block had its own water supply line from its block head box and water flow to the tanks ranged from 1.4 to 1.5 L/min. Compressed air was supplied to each tank via a commercial air stone (~13 cm long).

Two hundred and forty fish (15–35 g) were then randomly allocated, 1 fish at a time, to the 12 experimental tanks. Individual fish were weighed in water on a large animal balance (Sartorius LC34000P; Goettingen, Germany) to the nearest 0.1 g before allocation. Average fish weight in each tank ranged from 19.1 to 22.3 g at the beginning of experimental tank acclimation (Table 1). Light intensity above the experimental tanks was measured and adjusted to 1–2 lux (Traceable Dual Display Light Meter, Control Company; Friendswood, Texas, USA). Fluorescent lighting was used only occasionally but never within 1 hour of feeding.

On the day after termination of dosing (day 20), all fish in the experimental tanks (20 per tank) were immobilized by immersion in an ice bath (one tank at a time) then euthanized by cervical severance (7, 8). After fish from a tank were immobilized, the fish were indiscriminately removed from the ice bath and assigned a number from 1 to 20, weighed, and their total length recorded. Ten fish per tank were randomly selected for gross necropsy and histological sample collection. Concurrently, the remaining channel catfish were examined for gross external lesions only. A full necropsy was performed on 2 additional channel catfish with external lesions present at necropsy (these fish were not initially included in the random selection). The pathologist conducting the gross pathological exam was unaware of the treatment assignment when gross necropsies were performed. After gross necropsies were performed, the pathologist received a copy of the treatment code before histological examination of tissues. Tissues collected from the fully necropsied channel catfish included liver, anterior (head) kidney, posterior (trunk) kidney, spleen, heart, gill, skin with muscle, brain, eye, stomach, pyloric intestine, and rectal intestine. Tissues and residual carcasses were preserved in 10% neutral buffered formalin (1 fish per container with approximately 750 mL of formalin).

Using routine histologic methods, tissues were processed to paraffin blocks. Initially, only tissues from fish in the 0× and 5× dose groups were processed to hematoxylin and eosin-stained microscope slides. Microscopic examination was performed on all tissue sections collected from 20% of

TABLE 1.—Mean weight (n = 20; standard deviation in parentheses) of channel catfish determined at transfer to the experimental tanks and at terminal sampling.

Dose (mg/kg BW/day)	Tank	Transfer to experimental tanks		Predicted mean final weight by tank, g	Terminal sampling			
		Mean weight by tank, g	Mean weight by dose level, g		Mean weight by tank, g	Mean weight by dose level, g	Weight change, g[a]	Daily growth rate, g/day[b]
0	A1	19.1 (2.5)	20.3 (3.6)	24.0	24.2 (4.2)	26.7 (6.0)	5.1	0.15
	B4	22.3 (3.8)		27.2	29.3 (6.2)		7.0	0.21
	C2	19.5 (3.7)		24.4	26.7 (6.6)		7.2	0.21
10	A3	19.9 (3.6)	20.9 (4.4)	24.8	25.7 (5.1)	27.9 (6.4)	5.8	0.17
	B2	22.2 (4.5)		27.1	29.8 (5.9)		7.6	0.22
	C3	20.5 (5.0)		25.4	28.3 (7.5)		7.8	0.23
30	A2	20.7 (4.4)	20.7 (4.6)	25.6	26.1 (7.0)	26.5 (6.5)	5.4	0.16
	B3	21.8 (5.5)		26.7	28.5 (7.4)		6.7	0.20
	C1	19.7 (3.6)		24.6	24.9 (4.6)		5.2	0.15
50	A4	21.1 (4.5)	21.0 (4.0)	26.0	26.8 (6.3)	25.8 (5.5)	5.7	0.17
	B1	20.3 (3.5)		25.2	25.0 (5.4)		4.7	0.14
	C4	21.4 (4.0)		26.3	25.5 (5.0)		4.1	0.12

[a] Weight change (g) = Mean final weight (g) − Mean initial weight (g).
[b] Daily growth rate (g/day) = Weight change/34 days (number of acclimation and dosing days).

the fully necropsied channel catfish in each of the 0× and 5× dose groups. For the remaining 80% of the fully necropsied channel catfish in the 0× and 5× dose groups, only gill, liver, anterior kidney, and posterior kidney were initially examined. Based upon evidence of decreased hematopoietic/lymphopoietic tissue (H&L tissue) in Aquaflor-treated channel catfish of the 5× dose group compared to the 0× dose group, sections of spleen from all channel catfish of the 0× and 5× dose groups were subsequently examined. In addition, all anterior kidneys, posterior kidneys, and spleens from fish of the 1× and 3× dose groups were processed to slides and examined. To improve consistency in the severity grading of decreased H&L tissue, all of the collected anterior and posterior kidneys and spleens were ultimately reexamined in a blinded fashion (ie, the pathologist had no knowledge of the treatment groups). Reported results for the severity and incidence of decreased H&L tissue were based solely on this reexamination. The severities of inflammatory, degenerative, and proliferative changes were graded on a scale of 1 to 5 (1 = minimal, 2 = mild, 3 = moderate, 4 = moderately-severe, and 5 = severe).

Feed Preparation and Evaluation

Four 1,000-pound batches of basal rations (35% Fry II crumble [an approximately a one-eighth-inch pellet], Delta Western Research Center, Indianola, Mississippi, USA) were prepared by incorporation of the commercially available Aquaflor formulation (Aquaflor 50% Type A Medicated Article, 50% w/w; Schering-Plough Animal Health, Union, New Jersey, USA) into the feed mash prior to extrusion. Appropriate amounts of Aquaflor were added to the mash to achieve florfenicol concentrations of 0, 0.5, 1.5, and 2.5 g/kg feed. These concentrations provided nominal florfenicol dose rates of 0, 10, 30, and 50 mg/kg BW when fed at a rate of 2% BW/day. Feed was stored at room temperature until fed. A sample of the unmedicated control diet was analyzed for nutrient content, heavy metals, semivolatile organic compounds, and pesticides (Woodson-Tenent Laboratories, Inc, Memphis, Tennessee, USA). Feed nutrient composition was characterized as follows: moisture = 6.24 to 6.98%, protein = 36.97 to 39.95%, crude fiber = 3.7 to 4.1, carbohydrates = 41.1 to 42%, and crude fat = 5.3 to 7.3%. Contaminants were not detected.

The concentration of florfenicol in channel catfish feed was determined before (dosing day 0) and after dosing (dosing day 19) using a high-performance liquid chromatography (HPLC) method (30). The method limit of quantitation (LOQ) was reported to be 0.004 g/kg feed (30). Florfenicol concentration in feed on dosing day 0 vs dosing day 19 (Table 2) was analyzed by analysis of variance (ANOVA) using the dosing day as a classification variable and the nominal concentration as a by-variable. Before comparison, we confirmed that the data met the assumptions of normal distribution (Shapiro-Wilk statistic; 28) and equal variance (visual review of residual versus predicted plot). Unadjusted least-square means were used to compare florfenicol concentration between dosing days. Significant differences in the florfenicol concentration between dosing day 0 and 19 were not identified for any of the 3 medicated feed levels ($p > 0.05$, Table 2).

TABLE 2.—Mean florfenicol concentration determined by HPLC in feed samples collected on dosing day 0 and dosing day 19. Mean concentrations with the same letter are not significantly different ($p \leq 0.05$).

Dose, mg/kg BW/day	Feed nominal concentration, g/kg	Mean concentration, g/kg (standard deviation)		Percent of nominal concentration
		Day 0	Day 19	
Control	0	<LOQ[1]	<LOQ	NA
10	0.5	0.462[a] (0.0027)	0.461[a] (0.0028)	92.3%
30	1.5	1.45[a] (0.0042)	1.44[a] (0.0020)	96.3%
50	2.5	2.12[a] (0.0050)	2.12[a] (0.0053)	84.8%

[1] <LOQ = less than the Limit of Quantification (0.004 g/kg).

Feed Consumption and Estimated Dose Consumed

Tank outlets were protected with a fine mesh screen (15 × 18 lines per inch) to trap uneaten feed. Uneaten pellets were collected daily during acclimation and dosing by siphon into preweighed screened container (stainless steel mesh; 40 lines per inch) approximately 1 hour after feeding. Pellet weight loss from immersion in water was determined using a modified version of AOAC method 934.01 (2). The uneaten collected feed was dried at 74 to 85°C for 3 hours then weighed to the nearest 0.01 g. The original amount of uneaten feed was estimated by dividing the dry weight of uneaten feed collected by a correction factor (0.7437; MP Gaikowski, unpublished data) to estimate the actual amount of offered feed that was not eaten.

The florfenicol daily dose was calculated based on (1) the mean feed florfenicol concentration (Table 2), (2) the estimated amount of feed consumed daily, and (3) the daily mean biomass in the tank (based on the daily growth rate; mean final weight (g) − mean initial weight (g))/34 d). We assumed that the amount of feed eaten was equal among fish within an experimental tank. The total dose of florfenicol administered for each tank was calculated by totaling the estimated daily delivered doses.

Behavioral Observations

During the treatment periods, fish in experimental tanks were observed daily for feed consumption and for changes in behavior and flight/fright response. Technicians assessing behavior had no knowledge of the treatments assigned. Fish distribution within the experimental tanks was recorded after incandescent lighting was on but before fluorescent lights were turned on and before tank cleaning. Fish distribution was defined as: (1) Dispersed (fish generally dispersed throughout the tank); (2) Crowded (fish generally crowded at the water flow inlet); or (3) Podded (fish generally congregated or "podding" in a shaded area of the tank).

The flight/fright response of fish within the experimental tanks was recorded following or concurrently with fish distribution. The flight/fright response was assessed by slowly waving an arm over each tank before cleaning. The flight/fright response was categorized as follows: (1) Lethargic (fish seem unresponsive with little to no flight/fright response); (2) Normal (fish are dispersed throughout the water column and react to movement by podding or rapid movement away from the movement, then appear to calm relatively

quickly); or (3) Frantic (fish make erratic bursts to evade the movement source, seem to "bounce off the walls").

Feeding activity was recorded concurrently or shortly after offering feed. When more than half the food was consumed, the tank was assigned a numerical score of 2. When less than or equal to half the food was consumed, the tank was assigned a numerical score of 1. Those tanks whose fish ate poorly or not at all were assigned a numerical score of 0.

Behavioral observations were recorded according to the methods previously described on each day during experimental tank acclimation. Fish were generally dispersed throughout the water column during acclimation except that podding was occasionally observed. Fish exhibited a normal flight/fright response to observer stimulus throughout acclimation. Feeding activity increased as fish were acclimated to the experimental tanks, starting at an activity level of 0 on the first 2 days of acclimation and then reaching an activity level of 2 on acclimation days 10 through 13.

Water Chemistry

The temperature in the experimental tanks was measured with a calibrated thermocouple (Barnant Model 115, Barnant Co, Barrington, Illinois, USA) and ranged from 26.3 to 27.2°C during acclimation and 26.3 to 29.0°C during dosing. Dissolved oxygen was measured with a YSI Model 55/12 FT (Yellow Springs Instruments, Inc, Yellow Springs, Ohio, USA) and ranged from 6.2 to 8.6 mg/L during acclimation and 6.0 to 8.2 mg/L during dosing. The pH of experimental tank water was measured with a Beckman Φ210 pH meter (Beckman Instruments, Inc., Fullerton, California, USA) and ranged from 7.70 to 8.32 during acclimation and from 8.08 to 8.12 during dosing. Alkalinity and hardness were measured titrimetrically (3). Alkalinity ranged from 112 to 132 mg/L as $CaCO_3$ and hardness ranged from 152 to 168 mg/L as $CaCO_3$. No potential contaminants were identified in water samples collected on dosing day 0 and 19 and analyzed for heavy metals, volatile organic compounds, and semivolatile organic compounds and pesticides (Davy Laboratories, La Crosse, Wisconsin, USA).

Data Analysis

Continuous variables (feed consumption and growth) were analyzed by Mixed Model ANOVA, using tank (for feed consumption) or fish nested within tank (for growth) as the experimental unit (27). To control for the potential of a variance component for any random variable equaling zero in the mixed model, the degrees of freedom were automatically adjusted by the Satterthwaite option so that it was not necessary to drop the random term and refit the statistical model. Least squares means were run to compare growth and feed consumption at each dose versus control using the conservative Dunnett's adjustment for multiplicity. Contrast statements were used to evaluate linear trends in growth and feed consumption among all doses simultaneously. A significance level of $p < 0.10$ was used for all comparisons. Choosing this conservative significance level enhanced our ability to identify potentially subtle deleterious side effects associated with drug use.

The presence/nonpresence of a lesion was assumed to fit a binomial distribution; therefore histopathology data were analyzed by logistic regression in a General Linear Mixed Model (SAS GLIMMIX macro; 27) using fish (the observation unit) nested within tank (the experimental unit). Block and tank (denoted by block*treatment) were random variables. Tank proportion, the proportion of fish in a tank with a particular pathological lesion, was the response variable. The Kenward-Roger approximation for denominator degrees of freedom was used along with a random statement to allow for variability in the blocks. The block*treatment interaction term was used to approximate the tank error term. The output from the model was used to find the estimated proportions of lesion presence, as well as confidence intervals about each estimated proportion. A contrast statement was used to evaluate linear trends across all doses simultaneously. Least squares means were computed and used to compare the lesion tank proportion for each dose versus the control using Dunnett's adjustment with 90% confidence limits ($\alpha = 0.1$).

Good Laboratory Practices

All phases of this study were conducted in compliance with US Food and Drug Administration guidelines for Good Laboratory Practice Standards (21 CFR 58) except that the facilities that (1) prepared the feed, (2) analyzed the feed for nutrients and possible contaminants, and (3) analyzed the water for possible contaminants were non-GLP-compliant facilities at the time the study was conducted.

RESULTS

Adverse Reactions and Mortality

Other than a decrease in the amount of feed consumed by the 30 and 50 mg/kg BW/day treatment groups, no grossly observable adverse reactions were observed during the in-life (acclimation and dosing) phase of the study. No fish died during treatment nor did any fish exhibit any signs of morbidity during the treatment period.

Feed Consumption and Dosing

The estimated daily dose of florfenicol and the estimated daily feed consumption data are summarized in Figures 1A–D. Florfenicol was not detected in the control diet, therefore control fish did not consume florfenicol during dosing. Fish were estimated to receive a mean daily dose of 8.5 (Figure 1B), 24.6 (Figure 1C), and 34.9 mg/kg BW/day (Figure 1D) when dosed at nominal dose rates of 10, 30, and 50 mg/kg BW/day, respectively. The total delivered dose (average delivered dose × 20 d) was estimated to range from 168.2 to 170.9 mg/kg BW for the 10 mg/kg BW group, 468.5 to 516.6 mg/kg BW for the 30 mg/kg BW group, and 665.1 to 736.0 mg/kg BW for the 50 mg/kg BW group. The total delivered dose represented multiples of 1.7, 4.9, and 7.4 times the recommended total dose of 100 mg/kg BW (10 mg/kg BW/day × 10 day).

The statistical model that included classification terms for nominal dose, dosing day, and their interaction term and random term of block*nominal dose adequately described the variation in feed consumption. Feed consumption among the 4 dose levels was initially quite similar (dosing day 0 through dosing day 10; Figures 1A–D). However, feed consumption by fish dosed at 30 and 50 mg/kg BW/day (Figures 1C and 1D) was significantly reduced beginning on dosing day 14 (the 15th day of dosing) compared to controls and fish dosed at 10 mg/kg BW/day (Figures 1A–B; $p < 0.10$). Notable

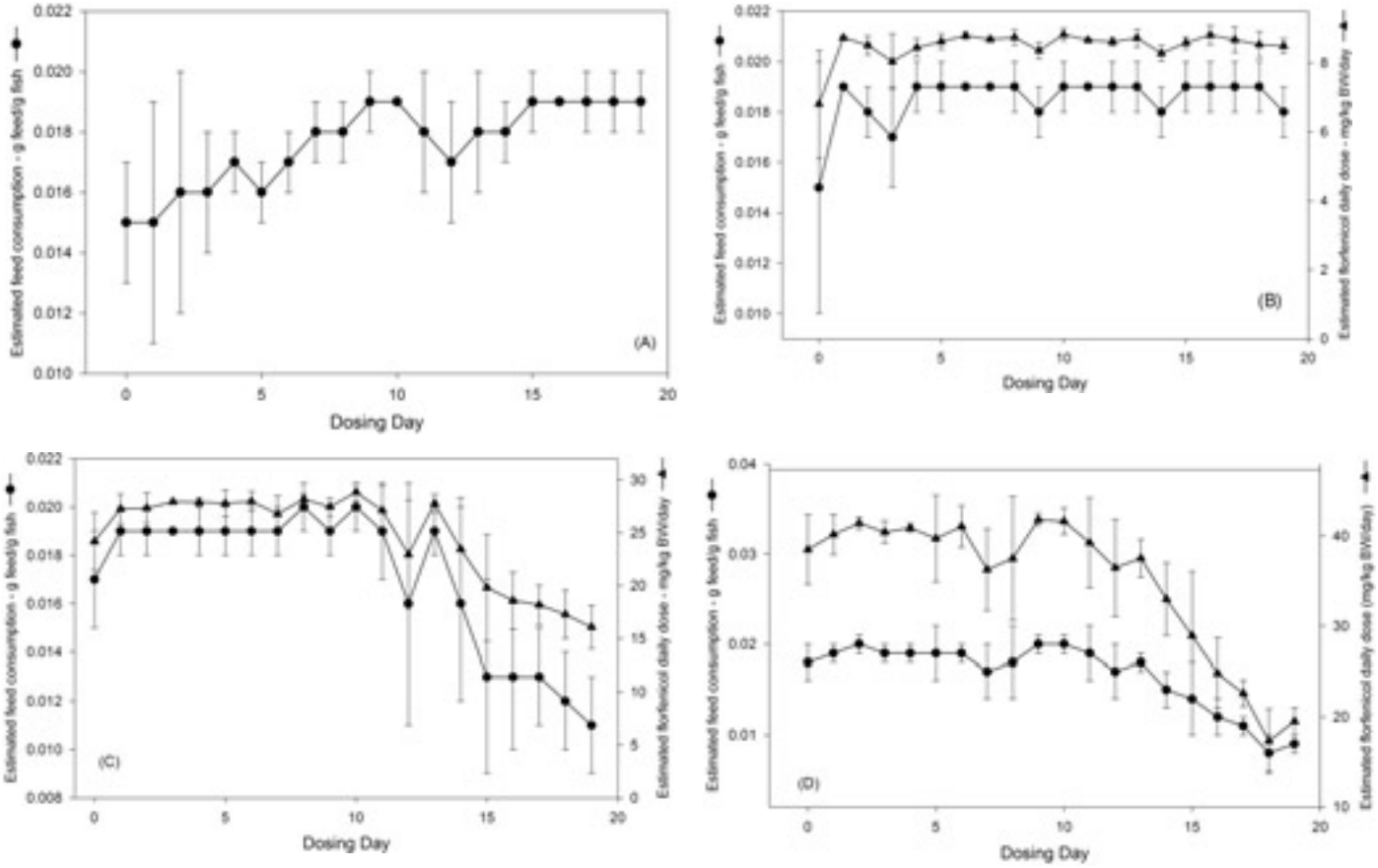

FIGURE 1.—Daily feed consumption of Aquaflor medicated feed and estimated daily florfenicol dose of channel catfish offered feed at nominal dose rates of 0 (A), 10 (B), 30 (C), or 50 (D) mg/kg body weight (BW)/day for 20 days. Solid circles represent the mean daily feed consumption (g feed consumed/g fish) and solid triangles represent the estimated daily florfenicol dose (mg/kg BW/day), error bars around each symbol represent the 95% confidence intervals.

within-dose decreases in feed consumption occurred in the 30 and 50 mg/kg BW/day dose groups from dosing day 11 through 15 (30 mg/kg BW/day; Figure 1C) and dosing day 13 through 18 (50 mg/kg BW/day; Figure 1D).

Clinical Observations

No consistent dose-related changes in appetite, distribution, or flight/fright response were observed. Assessment of fish feeding behavior initially showed good agreement with the amount of feed recovered but became a poor indicator of feed consumption at the end of the study when appetite was at a level 2 but the amount of uneaten feed increased in the 30 and 50 mg/kg BW/day groups (Figures 1C–D).

Growth

Fish mass increased over the 34 days within the experimental tanks (14 acclimation days + 20 dosing days). The average weight (by dose level) at the initiation of acclimation ranged from 20.3 to 21.0 g (Table 1). Average weight ranged from 25.8 to 27.9 g some 34 days later at terminal sampling (Table 1). Mixed model analysis indicated that there was no linear effect of dose level on fish growth (Table 1). Although there was no effect of dose on fish growth, there did appear to be an effect of tank location within the experimental room on growth. The tanks located nearest the entrance door (A1, A2, and A3) had consistently lower weight gains (Table 1) relative to other tanks at the same dose. This trend was reversed for tank A4, located in the back corner of block A. Tank A4 was relatively isolated from the entry door as well as from personnel movement during data collection. Mean weight of fish in tank A4 was 1.0 to 1.7 g greater than that of other fish dosed at 50 mg/kg body weight (Table 1).

Pathology

Aquaflor-related findings were limited to a histomorphologically evident, statistically significant, dose-dependent minimal to mild decrease in hematopoietic/lymphopoietic (H&L) tissue in the anterior kidney ($p < 0.01$, Figure 2A), posterior kidney ($p < 0.01$, Figure 2B) and spleen ($p = 0.02$, Figure 2C). Both the severity and prevalence of this finding increased as the dose of Aquaflor was increased and extended to the lowest florfenicol dose group (10 mg/kg BW/day). Hematopoetic/lymphopoetic tissue (Figures 3A, 3C, and 3E) was identified as aggregates of small round cells with dark basophilic nuclei and minimal cytoplasm (consistent with immature erythroid cells and/or lymphocytes), admixed with cells that featured larger, paler, and less round nuclei (myeloid and erythroid precursors). In affected fish (Figures 3B, 3D, and 3F), the interstitial areas of the kidneys and the areas surrounding the splenic periarteriolar sheaths clearly contained comparatively fewer and smaller H&L aggregates when compared to the majority of control fish.

In the anterior and posterior kidneys of affected fish, increased numbers of nonhematopoietic cells within the interstitium tended to compensate for the tissue decrease (Figures 3B and 3D). These nonhematopoietic interstitial cells were characterized by small, dense oval, elongated, or crescent-shaped eccentric nuclei and moderate amounts of pink cytoplasm. As such, the interstitial cells generally resembled the interrenal cells (analogous to adrenal gland tissue in mammals) that were also present to a lesser degree within the interstitium of unaffected control kidneys (11). Due to the nonspecific appearance and location of the interstitial cells, it is also possible that at least some proportion of these cells were dendritic stromal cells and/or mononuclear phagocytes. Unlike the kidneys, a compensatory increase in interstitial tissue was not observed in affected spleens (Figure 3F).

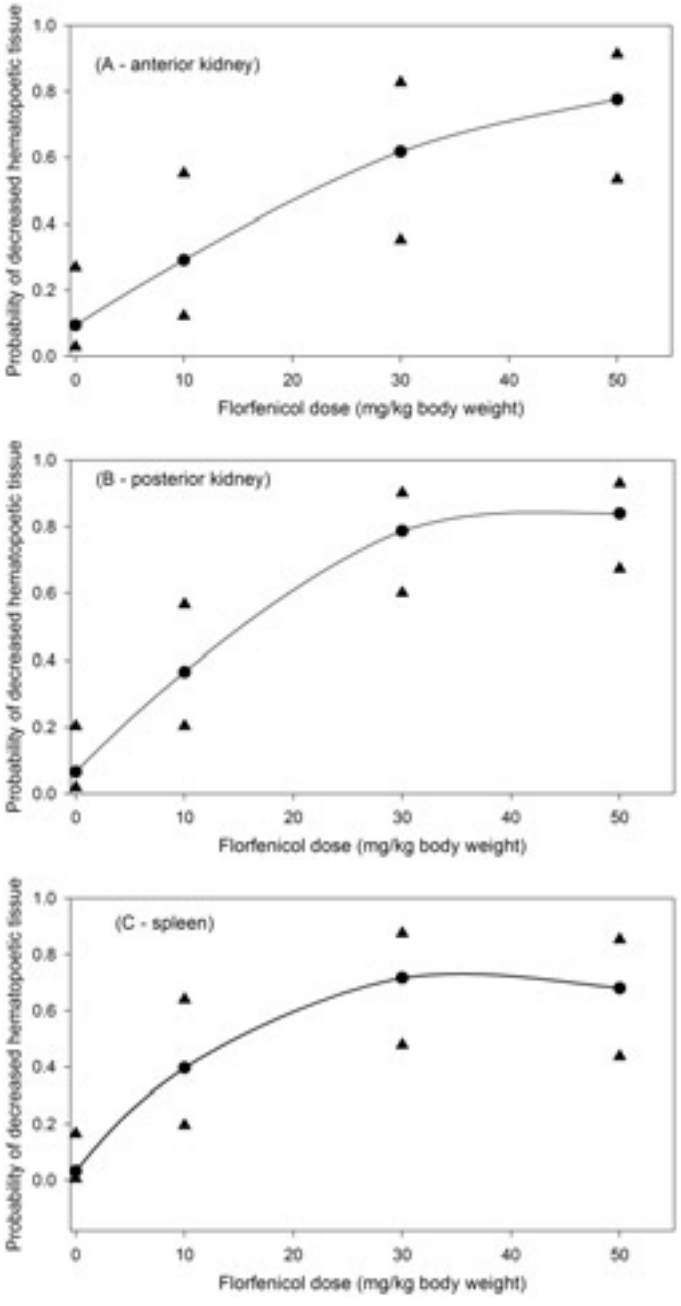

FIGURE 2.—The probability of decreased hematopoietic/lymphopoetic tissue in the anterior kidney (A), posterior kidney (B), and spleen (C) of channel catfish offered florfenicol medicated feed for 20 days and selected dose rates. Solid circles represent the predicted mean probability, 95% confidence intervals are given by triangles.

DISCUSSION

Aquaflor therapy in channel catfish induced a minimal to mild dose-dependent decrease in hematopoietic/lymphopoietic tissue in the anterior and posterior kidney, and spleen in the present study. Antibiotics are known to express effects on the immune system of several fish species. In vitro (carp *Cyprinus carpio* fry; 31) and in vivo studies (rainbow trout; 20, 22) showed florfenicol-induced suppression of immune response. In vitro macrophage phagocytic ability was suppressed following florfenicol treatment of carp and rainbow trout (20, 31). Lunden et al. (22) reported that in vivo macrophage phagocytic ability was suppressed in rainbow trout following florfenicol treatment. Carp and rainbow trout head kidney lymphoid cell proliferation following mitogen stimulation was reduced by in vitro florfenicol treatment (20, 31). Both oxolinic acid and oxytetracycline suppressed components of the humoral and cellular immune response of carp and rainbow trout (12, 21, 26). Unlike oxolinic acid or oxytetracycline, florfenicol therapy did not suppress circulating leucocyte levels nor antibody production in rainbow trout (21, 22). Florfenicol treatment of rainbow trout concomitant with vaccination with a divalent vaccine (an antigen stimulus) did not suppress antibody production nor circulating leucocytes (22) whereas florfenicol therapy in the absence of antigen stimulus suppressed the mitogenic response of rainbow trout head kidney cells (20).

In the present study, the treatment-related minimal to mild decreased hematopoetic/lymphopoetic tissue in the kidney and spleen could have been caused by decreased proliferation and/or increased destruction of H&L tissue. Our histopathological examination, however, did not indicate any overt evidence of tissue destruction as there were no increased karyorrhexis, pyknosis, or cellular debris in either the kidney or spleen. In addition, there were no cytoplasmic vacuolation or toxic basophilia, two common toxin-induced changes observed cytologically in mammalian hematopoietic tissue. In a previous safety study, histopathological changes were not observed in the kidneys of Atlantic salmon *Salmo salar* parr dosed at up to 100 mg/kg body weight per day for 10 days (17). In contrast, toxic changes in the hematopoietic tissues were induced in laboratory rats and dogs at very high doses (200–300 mg/kg body weight) of amphenicol antibiotics (chloramphenicol, thiamphenicol, and florfenicol). The clinical manifestations of amphenicol-induced impairment of the myeloid function included decreased number of circulating white blood cells (especially neutrophils) and cytologic/histopathologic evidence of vacuolation, degeneration, necrosis, maturation arrest, and reduced cellularity (9, 18, 24, 36).

Since there were no microscopically evident changes associated with cell death in the current study, what would explain the minimally to mildly decreased H&L tissue observed in this study? One possible hypothesis is that florfenicol may

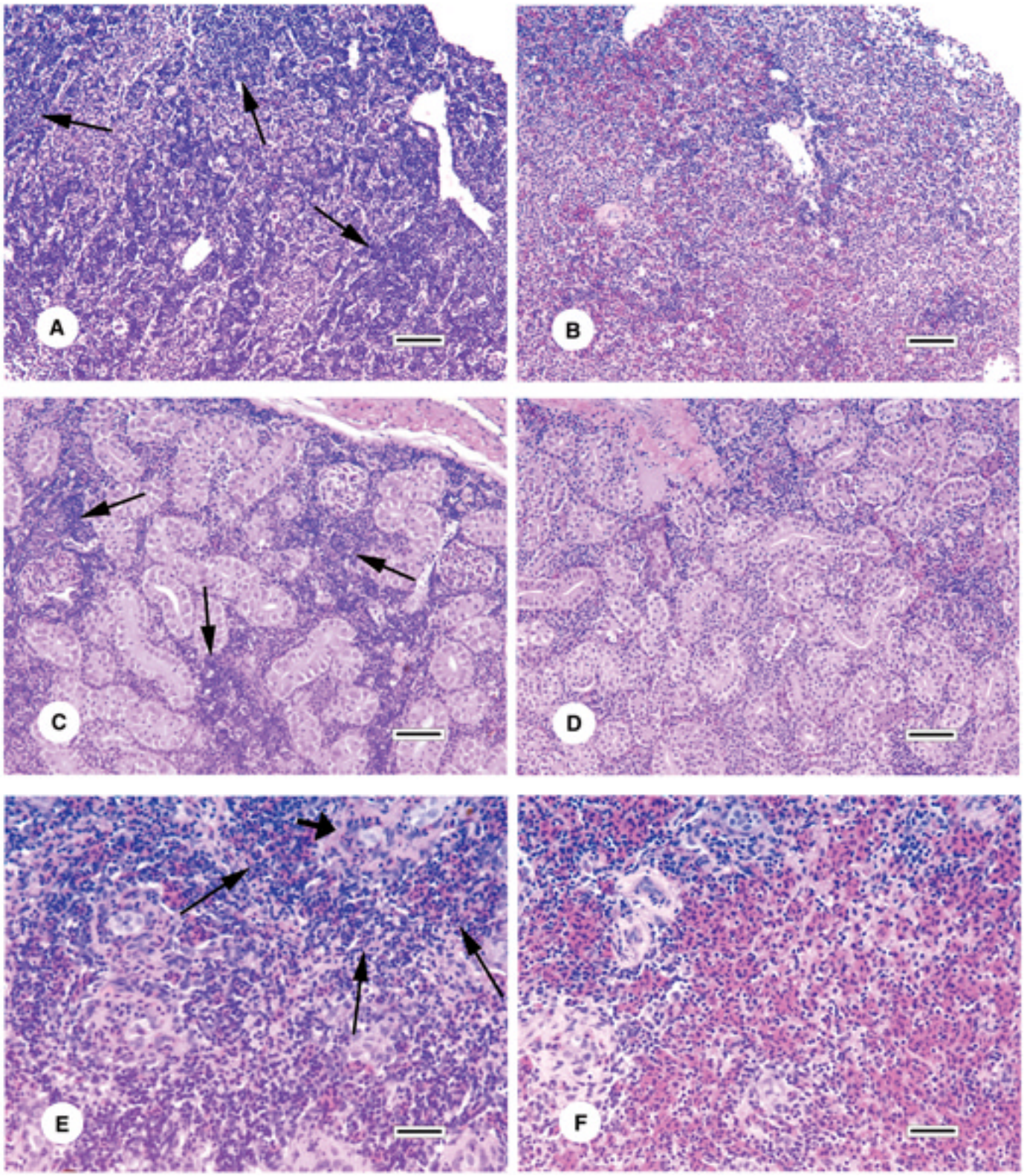

FIGURE 3.—(A) Anterior kidney from an untreated catfish. Arrows indicate the dense irregular cords of basophilic cells that comprise the normal hematopoietic/lymphopoietic tissue (H&L tissue). Bar = 50 μm. (B) Anterior kidney from a catfish treated with the 5× dose of florfenicol. The amount of H&L tissue is decreased. Bar = 50 μm. (C) Posterior kidney from the same untreated catfish as in 3A. Arrows indicate the normal H&L tissue that is situated between the renal tubules. Bar = 50 μm. (D) Posterior kidney from the same 5× dose catfish as in 3B. There is partial depletion of the H&L tissue. Bar = 50 μm. (E) Spleen from the same untreated catfish as in 3A and 3C. A splenic ellipsoid (small arrow) is encircled by a zone of H&L tissue (large arrows). Bar = 25 μm. (F) Spleen from the same 5× dose catfish as in 3B and 3D. Splenic ellipsoids are surrounded predominately by red blood cells (ie, H&L tissue is decreased). Bar = 25 μm.

impair cellular energy production in these tissues. Several antibiotics, including florfenicol, are known to accumulate in the kidney of teleosts (13, 15). Amphenicol antibiotics exert their antibacterial effect by binding to the 70S ribosomes (50S subunit) of prokaryotic cells and inhibit protein production (6, 23, 37), eukaryotic cells have 70S ribosomes in the mitochondria. Amphenicol antibiotics are known to inhibit heme synthesis in erythroid mitochondria (1, 9, 38) as well as colony stimulating factor on myeloid precursors in mammals. Alternatively, decreased H&L tissue in florfenicol-treated catfish could reflect the antibacterial effect of the antibiotic; ie, it is possible that reduced antigen stimulation due to reduced bacterial challenge was responsible for a decrease in lymphopoetic tissue rather than a direct toxic action of the antibiotic. Germ-free rats and mice have lower circulating leukocytes, below the historical control range, than their specific-pathogen free (SPF) counterparts in the same laboratory. In addition, germ-free rodents have lower cellularity in the thymus, lymph nodes, spleen and/or bone marrow than their SPF counterparts (5, 14, 16, 19, 33).

Unfortunately, studies that would support either of these hypotheses have not been performed in fish, therefore the cause of the decreased H&L tissue we observed in channel catfish cannot be definitively determined. Assessing lymphopoeisis or erythropoesis may have provided insight as to the clinical significance of the reduced H&L tissue. However, those data were not collected, nor did our study design allow us to determine whether histomophological changes were transient because the fish were necropsied immediately after administration of florfenicol-medicated feed.

There are insufficient data in the present study to determine if the decrease in H&L tissue that was associated with florfenicol administration is an adverse effect. Florfenicol, has been used extensively for the control of systemic bacterial diseases in salmonids (in Japan since 1990) without adverse affect (29) and did not induce histopathological changes in Atlantic salmon at up to 10 times the recommended dose (17). Given the pathogenicity of the bacteria frequently controlled by florfenicol treatment and the lack of apparent adverse effects in field trials, the relative risk of minimal to mild, and possibly transient, decreases in H&L tissue may be outweighed by the disease control that is currently realized.

CONCLUSIONS

Channel catfish dosed with Aquaflor at the recommended therapy of 10 mg/kg BW/day for 10 days will not display dose-related changes in behavior (appetite, distribution within tanks, or fright/flight response), feed consumption, or growth. Aquaflor therapy in channel catfish may induce a minimal to mild dose-dependent decrease in hematopoietic/lymphopoietic tissue in the anterior and posterior kidney, and spleen.

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