Palatability and Efficacy of Aquaflor®/ Aquafen® (florfenicol) for Control of Enteric Septicemia of Catfish

KEY POINTS:

Palatability concerns with available products have fostered disease response strategies whereby feed is restricted during ESC outbreaks.

Mortality losses and the absence of feeding suspend all opportunity for continuing weight gains during an ESC disease event.

Multiple research studies show that Aquaflor is highly palatable and well tolerated by catfish.

Aquaflor provides excellent efficacy for controlling mortality due to ESC.

Aquaflor offers the opportunity to limit mortality losses and maintain weight gains during ESC outbreaks.

In terms of monetary losses, enteric septicemia of catfish (ESC) is the most important disease threat that catfish producers must confront. The disease is caused by a Gram-negative bacterium, *Edwardsiella ictaluri,* that often triggers high mortality rates in U.S. production facilities, with spring and autumn as the seasons of peak disease risk.

For many years, two feed-grade antimicrobial products have been used in catfish (in the U.S.): oxytetracycline (Terramycin™; Pfizer), and a combination of sulfadimethoxine and ormetoprim (Romet™; PharmAQ AS). These products, however, have some distinct drawbacks that limit their utility. Oxytetracycline is not approved for ESC, and is often formulated in sinking feeds rather than floating feeds. Poor consumption rates of Romet have been documented[1] and pose an obvious barrier to consistent efficacy. Furthermore, bacterial resistance to both products has been reported.[2,3]

Palatability concerns associated with Romet have fostered disease response strategies whereby feed is restricted during an ESC outbreak, with resumption of feeding only after the disease event has resolved. While this strategy may make sense if the administration of medicated feed is viewed to offer little or no benefit, producers can suffer significant fish mortality losses due to uncontrolled disease, and the absence of feeding suspends all opportunity for continuing weight gains during the ESC outbreak. In addition, short-term feed deprivation has been demonstrated to reduce antibody responses in channel catfish, negatively influencing innate disease resistance.[4]

Fortunately, a new medication alternative for ESC has arrived for US catfish producers: Aquaflor,® from Schering-Plough Animal Health Corp. Aquaflor contains florfenicol (FFC), a potent broad-spectrum antimicrobial that has demonstrated high efficacy against a variety of fish pathogens. Aquaflor is widely used throughout the world for treatment of aquacultural diseases of both warm- and cold-water fishes, coupling excellent palatability with high efficacy. The product is FDA-approved for oral administration in catfish feeds at the rate of 10 mg FFC per kg body weight per day for 10 days, for the control of mortality in catfish due to enteric septicemia (ESC) of catfish associated with *Edwardsiella ictaluri.*

This *Bulletin* summarizes some of the extensive database documenting both the palatability and efficacy of Aquaflor in catfish, including research studies involving severe ESC disease.

Aquaflor® (florfenicol)

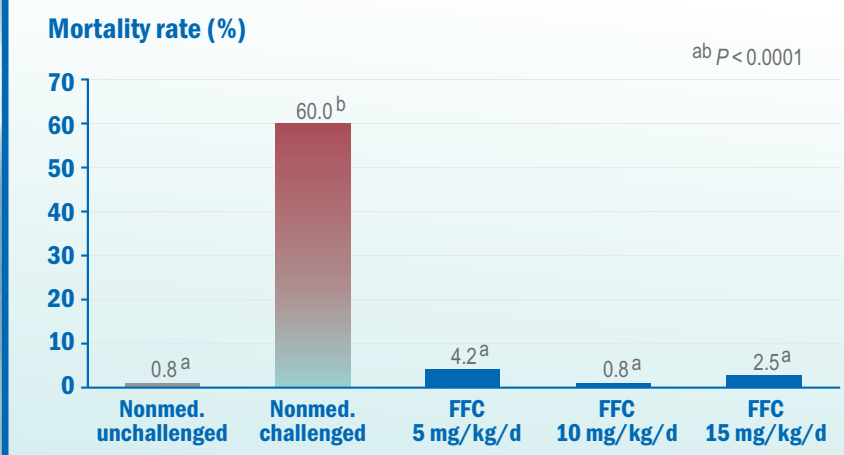
Palatability Studies

Several palatability and safety studies were conducted in channel catfish as part of the development program supporting FDA approval of Aquaflor in the US.

Dose Titration Study – A laboratory challenge study evaluated several different Aquaflor dose rates in the floating rations of fish with induced ESC disease.[5] Twenty catfish fingerlings were stocked in each of 30 tanks, and the tanks were randomly allotted to 5 treatment groups:

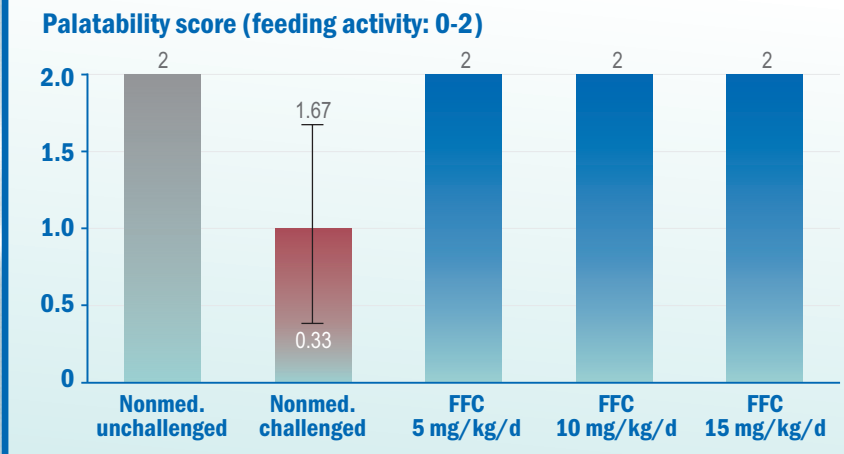
1) nonmedicated, nonchallenged controls;
2) challenged, nonmedicated controls;
3) challenged, plus Aquaflor at 5 mg FFC/kg/day for10 days;
4) challenged, plus Aquaflor at 10 mg FFC/kg/day for10 days;
5) challenged, plus Aquaflor at 15 mg FFC/kg/day for10 days.

FIGURE 1: Aquaflor (FFC) dose titration study.



Cumulative mortality of catfish challenged with *E. ictaluri* and fed floating feeds containing Aquaflor for 10 days vs. nonmedicated, fed controls.

FIGURE 2: Aquaflor (FFC) dose titration study.



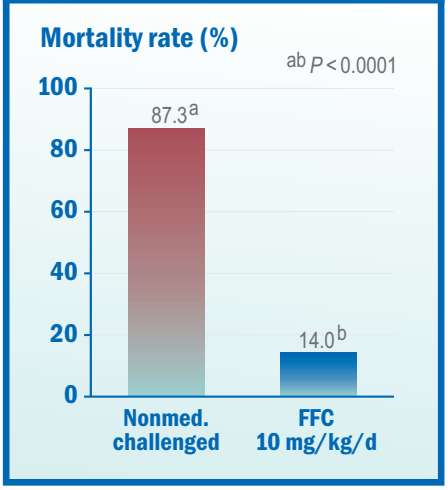
Average palatability score of catfish challenged with *E. ictaluri* and fed floating feeds containing Aquaflor for 10 days vs. nonmedicated, fed controls.

After a 10-day acclimation period in the tanks, fish were challenged with virulent *E. ictaluri* by immersion on day 0. Fish in all treatment groups were fed their respective treatments at the rate of 2.5% body weight for 10 consecutive days, with mortality as the primary response variable. Palatability of the floating feed was assessed using a numerical scoring system ranging from 0 to 2 (2=50-100% of feed consumed; 1=< 50% consumed; 0 = no food consumption). The challenge isolate had a florfenicol MIC of 0.25 μg/mL.

Results summarized in Figure 1 show that all Aquaflor dose rates significantly reduced mortality ($P < 0.0001$) compared to challenged controls. The severity of the challenge infection was demonstrated by a high cumulative mortality rate of 60% (range 45-85%) in the nonmedicated challenged treatment group. While all dose rates of Aquaflor produced high reductions in mortality, 10 mg FFC/kg/day was selected as the optimal Aquaflor dose based on other studies that determined the shedding time of *E. ictaluri*[6] and because feeding hierarchies exist in catfish populations.

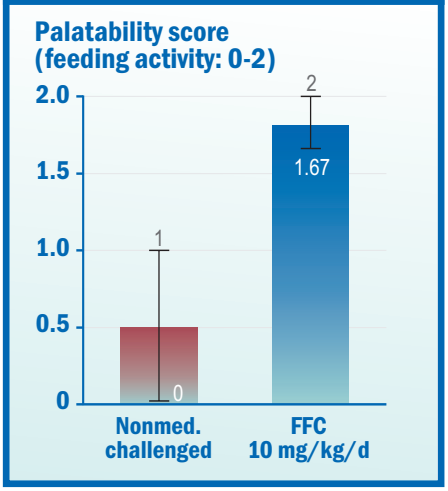
Excellent palatability was documented in all Aquaflor-treated fish and the unchallenged controls, with these groups maintaining a

FIGURE 3: Aquaflor (FFC) dose confirmation study.



Cumulative mortality of catfish challenged with *E. ictaluri* and fed floating feed containing Aquaflor for 10 days vs. nonmedicated, fed controls.

FIGURE 4: Aquaflor (FFC) dose confirmation study.



Average palatability score of catfish challenged with *E. ictaluri* and fed floating feed containing Aquaflor for 10 days vs. nonmedicated, fed controls.

feeding score of 2 (highest score possible) throughout the medication period (Figure 2). In contrast, feeding activity of the challenged controls ranged from 0.33 to 1.67, demonstrating the typical inappetence associated with acute ESC.

This study demonstrated that the recommended dose rate of Aquaflor (10 mg FFC/kg/day for 10 days) was readily consumed and highly effective for the treatment of acute ESC.

Dose Confirmation Study – A similar study evaluated the approved Aquaflor dose rate in fish with induced ESC disease.[5] Twenty catfish fingerlings were stocked in 30 tanks, and the tanks were randomly allotted to 2 treatment groups:

1) *E. ictaluri*-challenged, nonmedicated controls;
2) challenged, plus Aquaflor at 10 mg FFC/kg/day for10 days.

All other aspects of the study protocol were identical to the previous dose-titration study.

The results of the study are summarized in Figure 3. The mean cumulative mortality in the untreated control group was 87.3%, but mortality in the Aquaflor-medicated group was significantly reduced ($P < 0.0001$) to only 14%.

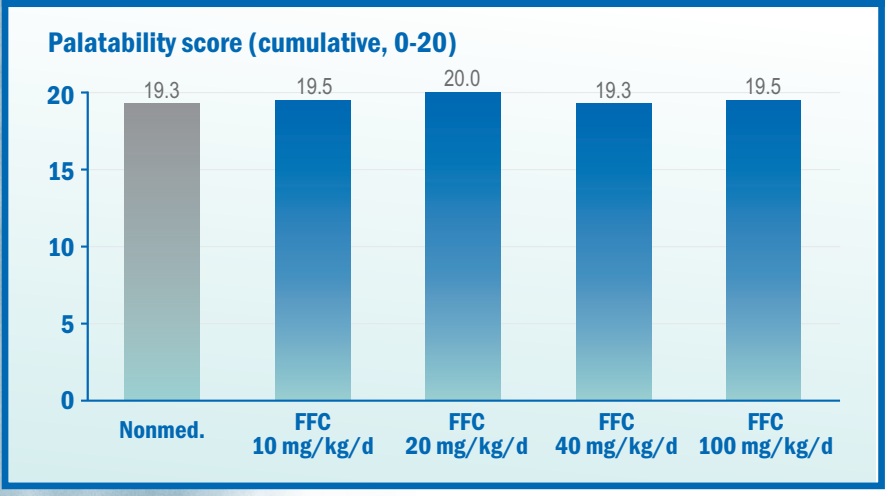
Excellent palatability of floating feed containing Aquaflor was again demonstrated, even though the ESC disease challenge was severe. The mean feeding activity score of the Aquaflor group ranged from 1.67 to 2 during treatment (Figure 4), compared with only 0 to 1 for controls. The lowest average consumption in each group was observed on day 4, with 1.67 and 0.13 for the Aquaflor and control groups, respectively.

This study confirmed that the recommended dose rate of Aquaflor (10 mg FFC/kg/day for 10 days) was readily consumed and highly effective for the treatment of ESC.

Palatability/Tolerance Study – A laboratory study was conducted to specifically evaluate the palatability of Aquaflor in catfish.[7] Twenty catfish fingerlings were stocked in each of 20 tanks, and the tanks were randomly allotted to 5 treatment groups. Aquaflor-medicated floating feed was offered to fish in 4 treatment groups, while the remaining group received nonmedicated feed (nonmedicated controls). One of the medicated groups received Aquaflor at the recommended dosage of 10 mg FFC/kg body weight/day for 10 days, while the 3 other groups received dosages of 20, 40, or 100 mg FFC/kg/day for 10 days, representing a significant degree of drug overdosage (2-, 4-, and 10-times the recommended dose rate, respectively). Fish were fed the experimental diets at 2.5% of their body weight daily, and their feeding behavior and feed consumption were recorded.

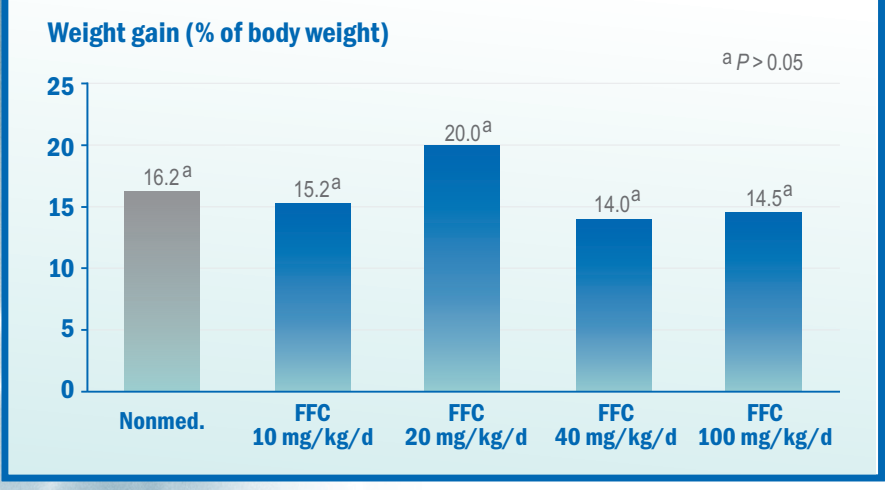
Palatability was assessed using the numerical scoring system as in the earlier studies (2=50-100% of feed consumed; 1=< 50% consumed; 0=no food consumption). A palatability score for each treatment group was computed as the

FIGURE 5: Aquaflor (FFC) palatability/tolerance study.



Cumulative palatability score (10 days) of catfish fed floating feed containing Aquaflor for 10 days vs. nonmedicated, fed controls.

FIGURE 6: Aquaflor (FFC) palatability/tolerance study.



Weight gain (as % of body weight) of catfish fed floating feed containing Aquaflor for 10 days vs. nonmedicated, fed controls.

sum of the average daily scores for the 10-day feeding period, so the maximum score for consumption of feed was 20. Fish weights were also recorded during the 10-day study.

Results summarized in Figure 5 demonstrate that all experimental diets were readily consumed by the catfish fingerlings. Even the treatment group receiving 10-times the recommended dosage of Aquaflor exhibited unhindered consumption of the medicated feed. In addition, weight gains were maintained in all treatment groups (Figure 6). No significant differences in weight gain ($P > 0.05$) were observed between treatment groups, indicating that Aquaflor overdoses of 2- to 10-times the recommended dosage did not impede growth. No differences in the histopathology of organs were observed in untreated and treated fish.

These results clearly demonstrate that diets containing Aquaflor are highly palatable and have no adverse impact on weight gains, even when the drug is overdosed.

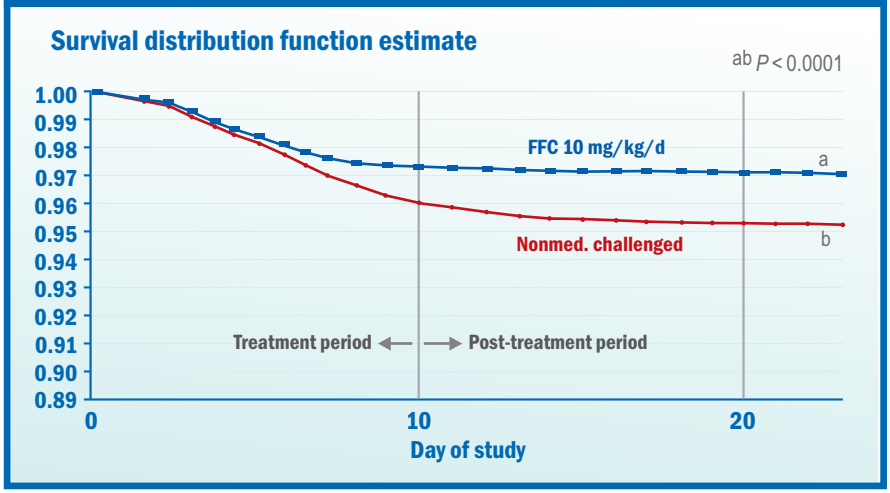
Pond Efficacy Study

A pond study was conducted to confirm the efficacy of Aquaflor for ESC control in catfish reared under commercial production conditions.[8] The study involved approximately 154,000 channel catfish fingerlings (150-180 days of age; 6.6-7.8 g) held in 14 ponds (about 0.1 acre each; approximately 11,000 fish/pond). The ponds were randomly assigned to 2 feeding treatment groups:

1) nonmedicated controls;
2) Aquaflor at 10 mg FFC/kg/day for10 days.

All fish were challenged with a pathogenic isolate of *E. ictaluri* obtained from a natural outbreak of ESC. Fish in all ponds were fed nonmedicated diets until the cumulative morbidity/mortality rate attributable to ESC reached 0.3% per pond. The 10-day medicated Aquaflor feeding regimen was then initiated in the ponds assigned to that treatment group. At the end of the treatment period, fish were observed for an additional 14 days. All ponds were monitored daily for morbidity and mortality, and dead fish were necropsied and

FIGURE 7: Survival analysis; Aquaflor (FFC) pond efficacy.



Kaplan-Meier survival analysis (treatment group strata) based on fish recovered after challenge with *E. ictaluri* and fed floating feed containing Aquaflor for 10 days vs. nonmedicated, fed controls (pond study). Odds ratio (2.20) indicates the odds of a mortality in the control group was 2.2-times the odds of a mortality in the FFC-treated group.

cultured for *E. ictaluri.* The challenge isolate had a florfenicol MIC of 0.25 μg/mL.

Results from the study were analyzed using survival analysis. Fish survival time was defined as the number of days a fish lived after the pond was admitted into the study. The survival analysis not only took into account a fish's living status at the end of the study (alive or dead), but also utilized the information of how long the fish lived. This allowed the survival rate of all the fish in the entire study period to be estimated and, therefore, provided a better understanding of the treatment effect over time.

Aquaflor proved effective in reducing mortality associated with the *E. ictaluri* challenge infection. Results from the survival analysis (based on fish recovered) are shown in Figure 7. The Aquaflor group had a significantly higher ($P < 0.0001$) survival rate than controls. Even though the disease severity was relatively mild, cumulative mortality rate in the Aquaflor group (3.05%) was significantly reduced ($P = 0.0397$) compared to controls (4.94%). Statistical analyses of these data yielded an odds ratio of 2.20 between treatment groups, meaning that the odds of a mortality in the control group was 2.2-times the odds of a mortality in the Aquaflor-treated group. No adverse reactions to treatment were observed.

Conclusions

A series of research studies have demonstrated that Aquaflor is highly efficacious and palatable. Floating feeds medicated with Aquaflor were readily consumed by catfish experiencing ESC outbreaks. Furthermore, feeds containing extreme overdosages of Aquaflor were still readily consumed by catfish, with medicated fish maintaining normal growth rates.

The reported sporadic palatability of some feed-grade antibiotic products has prompted some catfish producers to avoid use of medicated feed during outbreaks of ESC. When feed is withheld from fish during disease events, without supportive medication, mortality losses and the suspension of weight gains can seriously erode productivity and elevate production costs. In contrast, floating feed medicated with Aquaflor is highly palatable, even in fish clinically affected with ESC.

Aquaflor provides veterinarians and producers the opportunity to limit mortality losses and maintain weight gains during ESC outbreaks, thereby optimizing productivity and profit potential.

References

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For US users:

Caution: Federal law limits this drug to use under the professional supervision of a licensed veterinarian. Animal feeds bearing or containing this veterinary feed directive drug shall be fed to animals only by or upon a lawful veterinary feed directive issued by a licensed veterinarian in the course of the veterinarian's professional practice.

Specific product details such as indications, withdrawal time, etc., may vary by country. Please refer to your local package insert for details or contact your local Schering-Plough Animal Health representative.

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