Bacterial diseases can pose significant threats for aquaculture production regardless of the type of fish under management. Whether producing catfish in warm-water ponds, or trout or salmon in cold-water environments, an uncontrolled bacterial infection can quickly elevate mortality losses throughout the production unit, seriously eroding productivity and/or profitability.

When a disease outbreak is suspected, a rapid and reliable treatment response is needed to prevent devastating losses. The use of medicated feeds containing an antimicrobial agent effective against bacterial pathogens is usually the most cost-effective method for stemming a disease outbreak and restoring fish to health. However, the availability of antimicrobial agents approved for use in aquaculture is limited, bacterial resistance to some agents has been reported, and the successful incorporation of these agents into pelleted fish feeds can be challenging.

The manufacture of pelleted fish feeds typically employs production processes involving very high temperatures and steam. The extrusion process for the production of floating feeds, in particular, demands high temperatures at both the pelleting die and in the dryer (up to 300°F). Some antibiotic compounds used in aquaculture cannot survive these high temperatures and moisture conditions. For instance, oxytetracycline (i.e., Terramycin; Pfizer) is well known to degrade during high-temperature production processes, so it is usually available only in sinking feeds. The manufacture of pelleted feeds containing heat-sensitive compounds can demand extreme overages in order to produce feed pellets containing adequate amounts of drug. This greatly amplifies the cost of medicated feed production and reduces consistency and reliability of the finished feed product. Therefore, antimicrobial agents that can readily tolerate high production temperatures without significant degradation are beneficial for reducing feed production costs and ensuring drug content of the pelleted ration.

This Bulletin summarizes recent research regarding the feed pellet stability of Aquaflor, a novel antimicrobial approved for the control of bacterial diseases of fish.

**Aquaflor**

Aquaflor, from Merck Animal Health, is a feed-grade antimicrobial specially designed for aquaculture. Florfenicol, the active ingredient of Aquaflor, is a potent broad-spectrum antimicrobial that has demonstrated high efficacy against a variety of fish.

**Key Points:**

- Medicated feeds are usually the most cost-effective method for stemming outbreaks of bacterial disease and restoring fish to health.
- Some antibiotic compounds are degraded by high temperatures during the production of pelleted feeds.
- Aquaflor survives the pelleting process, with no significant losses during production of floating or sinking feeds.

For 5 test feeds, all batches exceeded 91% of the target florfenicol concentration and were well within the acceptance limits.

Aquaflor-medicated feeds remain stable for at least 4 to 13 weeks, depending on storage conditions.

**For safety information, please refer to the product label or Aquaflor VFD order form.**
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. Aquaflor can be added to fish feed by either incorporation prior to pelleting/extrusion or by top-dress. Aquaflor-medicated feed can either be floating or sinking. Aquaflor can be incorporated into a wide range of fish feeds and is available as a 50% premix approved in the U.S. for various indications in freshwater-reared finfish. The product is intended for oral administration to fish at the rate of 10 or 15 mg/kg florfenicol per kilogram of body weight per day for a 10 day treatment period.*

The proprietary manufacturing process used to produce Aquaflor promotes homogeneity in granulation, inert/binding agents, and the active ingredient. This helps ensure uniform drug administration to fish and enhances the exceptional stability of Aquaflor premix. All ingredients are tested for purity and manufactured in accordance with Good Manufacturing Practices (GMP) guidelines, with every batch analyzed for quality control. Aquaflor is highly palatable and can be used for fish in any stage of production. Furthermore, because Aquaflor can be used in top-dressed feed batches, any size batch can be produced (the size of pelleted feed batches is usually limited by mixer capacity).

### Experiment Design

A study was conducted to evaluate the stability, homogeneity, and tendency for segregation of Aquaflor in medicated fish feeds. The study involved 5 batches of Aquaflor-medicated feed manufactured at concentrations of 200 to 4000 ppm florfenicol (Table 1). Three of the batches were sinking salmonid feeds and 2 batches were floating catfish feeds. Of the 3 sinking feeds, 2 were treated with Aquaflor via a top-dress procedure while the remaining batch was prepared with Aquaflor incorporated in the pre-extrusion mixture. One of these top-dressed batches was prepared using an extrusion process and the other was prepared using an expansion process. The 2 floating catfish feed batches were both prepared by the incorporation of Aquaflor in the mash before extrusion. The acceptable batch assay limits were calculated according to FDA guidelines and vary depending on the individual characteristics of each batch (drug inclusion rates).

<table>
<thead>
<tr>
<th>Table 1</th>
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Production characteristics of 5 Aquaflor-medicated feed batches (3 sinking salmonid feeds and 2 floating catfish feeds).

<table>
<thead>
<tr>
<th>Feed</th>
<th>Florfenicol concentration (ppm)</th>
<th>Nominal pellet size</th>
<th>Method of manufacture</th>
<th>Medication method</th>
<th>Acceptable assay limits (% label claim)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonid A</td>
<td>200</td>
<td>2.4 mm</td>
<td>expanded</td>
<td>top-dress</td>
<td>80-110%</td>
</tr>
<tr>
<td>Salmonid B</td>
<td>4000</td>
<td>10.0 mm</td>
<td>extruded</td>
<td>top-dress</td>
<td>80-110%</td>
</tr>
<tr>
<td>Salmonid C</td>
<td>4000</td>
<td>3.0 mm</td>
<td>extruded</td>
<td>incorporated</td>
<td>80-110%</td>
</tr>
<tr>
<td>Catfish A</td>
<td>200</td>
<td>2.5 mm</td>
<td>extruded</td>
<td>incorporated</td>
<td>80-110%</td>
</tr>
<tr>
<td>Catfish B</td>
<td>4000</td>
<td>5.0 mm</td>
<td>extruded</td>
<td>incorporated</td>
<td>80-110%</td>
</tr>
</tbody>
</table>

* Food and Drug Administration (FDA) approved assay limits for all fish feeds. Assay specifications may vary by country.

* Freshwater-reared finfish: For control of mortality due to columnaris disease associated with *Flavobacterium columnare*; Freshwater-reared, warmwater finfish: For control of mortality due to streptococcal septicemia associated with *Streptococcus iniae*; Catfish: For control of mortality due to enteric septicemia associated with *Edwardsiella ictaluri*; Freshwater-reared salmonids: For control of mortality due to furunculosis associated with *Aeromonas salmonicida* and mortality due to coldwater disease associated with *Flavobacterium psychrophilum*. See package insert for details on approved doses and withdrawal times, by species and indication.
Aquaflor offers versatility for use in both extrusion and top-dress feed preparation methodologies. The extrusion process ensures uniform distribution of Aquaflor premix throughout the feed when the drug is added to the mash. The top-dressing method provides excellent distribution of Aquaflor premix in both wet and dry processes due to the uniform particle size of the premix and its even dispersion in oil.

A high-performance liquid chromatography (HPLC) assay was used to determine the concentration of florfenicol in feed samples. Ten samples were obtained at regular intervals at the bagging point in the feed mill and assayed to determine homogeneity of florfenicol within batches as well as the concentration of florfenicol that survived the pelleting feed-production process. To determine the tendency of florfenicol to segregate during the transportation of medicated feeds, samples were taken from the top, middle, and bottom of 2 bags randomly chosen from each batch before and after the bags were transported 50 miles (per FDA guidelines). These samples were then analyzed for florfenicol concentration.

To determine the long-term stability of florfenicol in finished feeds, 20 feed samples (500 g) from each batch were combined into 1 large mixture (10 kg) that was then divided into 500-g storage samples. These samples were stored in simulated commercial packaging at either 25°C with 60% relative humidity (RH), 40°C with ambient RH, or 40°C with 75% RH. The samples were analyzed for florfenicol content at multiple time points to determine the amount of degradation during storage for 4 to 13 weeks post-manufacture.

**Results**

**Homogeneity and Segregation**

All batches were found to be homogeneous within the calculated analytical acceptance limit of ±16.79%. After being transported 50 miles, the medicated feeds displayed no tendency for segregation in any of the 5 batches, regardless of where in the feed bags the samples were obtained.

**Stability**

Average assay results for the 5 batches of Aquaflor-mediated fish feeds immediately following production are summarized in Figure 1. All feed batches exceeded 91% of the target florfenicol concentration and were well within the acceptance limits. These results demonstrate that florfenicol readily
survives the pelleting production process, with no significant quantities of drug activity destroyed during the production of floating or sinking fish feeds regardless of the pelleting method or drug application process employed.

Stability of medicated feed was studied under accelerated conditions (40°C and either ambient or 75% RH) for 4 weeks, and under long-term storage conditions (25°C and 60% RH) for 13 weeks. The data from the study are presented in Figures 2 and 3 for the salmonid and catfish feeds, respectively. At 25°C and 60% RH, the rate of florfenicol degradation was not significant ($P > 0.05$) under pooled analyses. Upon individual analysis, 2 of the batches (salmonid C and catfish B) had detectable rates of degradation ($P < 0.05$); however, all batches were within the acceptance limits. The shelf life of Aquaflor-medicated feed under these storage conditions was determined to be at least 13 weeks (the duration of the study).

At 40°C and ambient RH, no significant ($P > 0.05$) florfenicol degradation for the combined batch analysis was observed, although the salmonid C batch showed significant ($P < 0.05$) degradation when individual batches were analyzed separately. All batches remained within acceptance limits. The shelf life of Aquaflor-medicated feed under these storage conditions was estimated to be at least 4 weeks (the duration of the study), consistent with regulatory requirements for accelerated storage conditions.

At 40°C and 75% RH, no significant ($P > 0.05$) degradation was observed for the combined batches. In 3 of the batches, individual analyses detected a significant ($P < 0.05$) rate of florfenicol degradation (salmonid C, catfish A and B). Still, all batches remained within acceptance limits. Again, the shelf life of Aquaflor-medicated feed at 40°C and 75% RH was estimated to be at least 4 weeks (the duration of the study), consistent with regulatory requirements for accelerated storage conditions.

To summarize, florfenicol-medicated pelleted feeds were shown to remain stable under accelerated storage conditions (40°C).
for 4 weeks, and under long-term storage conditions (25°C and 60% RH) for 13 weeks. Based on these results, there is no need to set an expiration date for feeds medicated with Aquaflor.

**Conclusions**

Five batches of pelleted fish feed containing Aquaflor were produced and tested for florfenicol stability, homogeneity, and tendency for segregation. No significant degradation of florfenicol concentration was observed after production of both floating and sinking pelleted fish feeds. In contrast to some other antimicrobials used in aquaculture, appropriate target drug concentrations of florfenicol were maintained in all tested feed formulations regardless of the manufacturing processes employed (extrusion or expansion, top-dress or incorporation). Furthermore, concentrations of active florfenicol in pelleted fish feeds remained stable and within acceptable boundaries at all storage conditions (25°C/60% RH, 40°C/ambient RH, and 40°C/75% RH) for all the storage periods tested (4 to 13 weeks).

Based on the analytical acceptance criteria, the 5 batches were considered homogeneous. No tendency for segregation was observed among any of the batches tested, regardless of where in the feed bag the sample was taken or which batch was evaluated.

These research results demonstrate that Aquaflor readily survives the pelleting production process and is highly stable in pelleted fish feeds, thus obviating the need for expiration dating of Aquaflor-medicated feeds in the US.
References


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 (VFD) drug shall be fed to animals only by or upon a lawful VFD issued by a licensed veterinarian in the course of the veterinarian’s professional practice. Extra-label use (i.e., use of the VFD feed in a manner other than as provided by the VFD drug approval) is strictly prohibited.