The following protocol is in a process of continual improvement and will be updated periodically.
Last update: 03-24-2021

Evaluating an Ingredient's effectiveness on Disease

Diseases and parasites pose a major threat to many sectors of the aquaculture industry. Disease and parasite challenge experiments evaluate a test ingredient's potential for improving fish immune responses and enhanced survival. In order to test an ingredient's efficacy against disease, it is important to condition the animals with different feeds several weeks in advance, prior to exposure to disease. There should be a two to three week pre-trial acclimation of animals brought into the lab from outside suppliers and a minimum of eight weeks feeding of the test diet.

While the seafood market is trending towards additive- and antibiotic-free products, some ingredient alternatives to fishmeal, such as black soldier fly hold promise for improved immunity. Flies thrive on waste with enhanced bacterial loads, and have genomes that reveal an enhanced immune system. Immunity enhances survival, increases fish conversion ratio, thereby improving the economics of a feed, making it a commercially important factor in feed choice.

One way to gain further insight in both growth and disease is to have researchers conduct a disease challenge after growth trials.

Considerations
When designing a disease trial, it is important to keep in mind several factors:

- What are the largest threats to the target aquaculture species, in terms of disease or parasites?
- What is the typical age of the species at which it is most vulnerable to infection? This age and/or size should be replicated in the experimental design.
- What is the most effective way to inoculate the positive test groups - i.e., cohabitation with an infected individual or direct inoculation via culture water, injection, or in feed?
- What is the quality assurance plan of the institution performing the disease trial? Experts should review the source of healthy, disease- or pathogen-free stock, and standard operating procedures for animal care.
- The responsible laboratory should possess the ability to determine the strain of the challenge organism and have prior history in disease management.
- Treatments should be randomly assigned to tanks or groups and a negative control group (one that is *not* challenged with the disease or parasite) should be included.

- Fish should be fed the same diet (one that is different from the trial diets) before the experiment begins. Prior to trial start and after trial end, peroxide values may be measured to give insight on potential rancidity issues with the feed, and to prevent or reveal a confounding variable.
- All treatment groups should follow the same feeding, inoculation, and data collection protocols.

Data Collection

Some data collection points will vary depending on the disease or parasite used in the trial. For instance, a sea lice challenge includes counting the number of lice present on each infested fish. However, the following metrics are typical of most disease challenge trials.

- Survival Rate (SR) - cumulative mortality (%) and relative percent survival RPS = [1 − (% mortality in challenged group/% mortality in control group)] × 100.
- Feed Consumption - daily and total
- Condition (K)
- Weight/length - individual
- Water quality - temperature, dissolved oxygen, salinity, pH, alkalinity, ammonia
- The F3 Team is conducting research on using fillet sample $\delta^{15}N$ isotope values to confirm dietary fishmeal substitution. If your lab has the funds to conduct this testing, please send your data to f3fishfreefeed@gmail.com. We appreciate your support!

Wherever possible/desirable to undertake hematological analysis, including immune response... For teleosts: Red blood cell (RBC) count, hematocrit (Ht), hemoglobin (Hb); respiratory burst activity of head kidney leukocytes; lysozyme assay (turbidimetric); superoxide dismutase (SOD) and myeloperoxidase (MPO) activity.

For shrimp: Total haemocyte count (THC); hyaline cell (HC), semigranular cell (SGC) and granular cell (GC) counts; hemolymph phenoloxidase (PO) activity.