



*The follow protocol is in a process of continual improvement and will be updated periodically.
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Palatability

This test is designed to determine the initial reaction of fish to the inclusion of a new ingredient that is being tested. It is extremely important to understand the effect of a new ingredient on feed intake (Glencross et al., 2007). It is possible for new ingredients to have a negative effect on the amount of feed consumed by fish simply because it is not desirable from a fish taste perspective or palatability. If feed palatability is greater, then fish will increase feed intake. To test palatability, a bland base diet should be used, which could resembles the control diet in Digestibility Protocol so that the new ingredient is the only new variable in the test, making it easier to determine the cause of any changes compared to control diet.

For palatability testing, it recommended to have 6 kg of the new ingredient available for feed production. The test diets should contain 70% of the control diet and 30% of the test ingredient. The inclusion rate of 30% is high but facilitates quick ingredient evaluation. The standard is to use extruded floating feeds to facilitate apparent satiation feeding. Feed preparation can occur through the testing facility or third party production. Where possible, the palatability study should be immediately followed by a digestibility study if the results of the palatability test are positive so that the entire testing is more cost-effective.

Rainbow trout and hybrid striped bass are recommended for palatability tests, and these guidelines are centered around these species. Fish should be 30g - 2 kg in size, since fish of this size are capable of ingesting floating feeds but not too large for the available tank sizes. For each of the diets, tested including control diets, the best practice is to use triplicate tanks (3), and a stocking density to allow for normal feeding behavior given the species and size of fish. Some species of fish will not feed normally if the stocking rate (number of fish per tank) is too high or too low. "Normal" feeding behavior can be a subjective observation, but it is best as expressed as the feed consumption rate that supports the expected growth rate based upon historical records. If slow growth and low feed intake is observed then higher or lower stocking densities should be considered.

For the first two weeks of the testing, all the tanks should receive the same diet to condition the fish. Hand feeding to apparent satiation 3-4 times per day is an accurate way to determine feed intake. For species that cannot be hand fed, a system that collects uneaten feed needs to be used to accurately determine palatability. The fish should be fed 7 days per week during palatability testing to reduce daily fluctuations in feed intake for two weeks.

After two weeks of feeding the control diet, the fish in test tanks should be fed the test ingredients containing the new ingredients for the following two weeks. The control groups should continue on the control diet. For instance, in the case there is one test ingredient, there would be 6 tanks total, triplicate for each diet group. During this period, feed intake is again measured for each tank and can be compared to the feed intake of fish that continued with the control diet after the two week conditioning period. Fish weight and size should be measured at the beginning and end of the trial. The average should be used to determine feed intake expressed as a percentage of body weight/day

Average body weight = (initial weight + final weight)/2

Average daily feed intake = total amount of feed fed / number of days

Feed intake per day = (average daily feed intake / average body weight) x 100

This test can determine if an ingredient has a positive or negative effect on feed intake during a palatability study. Note that the long-term effects of the ingredient is still considered unresolved due to the short study length.

For a conclusive assessment of long-term adaptability of fish to a new ingredient, a long-term test is needed. The procedures for long-term testing are outlined in section VII Grow-Out. Compared to palatability testing, long term feeding trials often include the test ingredient at various levels to determine any thresholds or optimal inclusion levels. If there is no significant difference ($P < 0.05$) between the trout fed the control diet and trout fed the test diet, it is considered a “pass” and the evaluation of the ingredient can go forward.

When there is a significant difference ($P < 0.05$) in feed intake between the fish fed the control diet and the test diet, the methods for processing the test ingredient should be reconsidered.

If the feed consumption is greater than 50%, it is advised to move forward in the process to determine long term feed adaptability (which may improve feed intake). If the feed consumption remains below 50%, the ingredient evaluation would be considered a fail. In the case of a fail, the producer would need to consider appropriate modifications to the ingredient should they want to pursue the aquafeed market.

Consultation is recommended with facility and nutrition experts for any other species-specific experimental design considerations.

References:

Glencross, B.D., Booth, M., Allan, G.L. A feed is only as good as its ingredients - a review of ingredient evaluation strategies for aquaculture feeds. *Aquaculture Nutrition* 13; 17-34. 2007