Protocols for evaluating Complete Diets 1 Mar 2018 DRAFT

By F3 (Future of Fish Feed) Team for the Feed Innovation Network (FIN)

Note that these Feed protocols are for the "grower diet" phase in fish production.

Why Evaluate Complete Diets?

In order to become more sustainable, feeds need to be formulated with more sustainable ingredients that still retain nutritional, physical and economic characteristics of the ingredients that they have replaced. The F3 (Future of Fish Feed) Team has created a set of protocols for ingredient evaluation for nutritional and physical characteristics. However, at the end of the day, an ingredient is included in a Feed that comprises a Complete Diet. Consequently, the entire diet, or Complete Diet, needs evaluation.

A Complete Diet evaluation is needed because in some cases the ingredients of the diet are unknown, but information about the diet efficacy is still desired.

This protocol outlines an approach to evaluate Feeds in their entirety or Complete Diets (CD). The goal of this protocol is to offer a complete evaluation of CDs so that their efficacy can be compared for different species, and establish baselines for comparison against other diets.

Definitions:

Complete Diet protocols compare performance of fish fed diets that simultaneously vary nutrients and ingredients. An ingredient may have nutritional and economic value in a prior Ingredient Evaluation, but once it is included in a feed, it is considered a Complete Diet (CD), since the performance of fish is affected by all the ingredients in the formula and the nutrients represented (i.e. crude protein, lysine, methionine etc.).

Open Formula Feeds: CDs composed of known ingredients, with known inclusion levels for each ingredient, are considered for the purposes of this paper, Open Formula feeds. Open Formula feeds are often developed by Academic Institutions and Government Research Agencies as tools for specific research projects or for use in Federal Hatcheries. Open Formulas allow direct comparison between feeds tested in different years and at different institutions because they can be fully replicated.

Closed Formula Feeds: By contrast, Feeds produced by commercial feed companies are most often Closed-Formula: the exact ingredient composition and inclusion levels are proprietary. Often, the formulation of commercial feeds will change

over time as feed producers introduce enhancements to nutrition and price. When multiple commercial diets are studied, fish farmers should realize that this is a "snapshot" in time since commercial feed companies could have changed their proprietary formula.

Reference Feeds: Commercial feeds are often used as 'Reference Feeds' since they provide a reference for what is commercially available at the time. A suitable reference feed should be commonly used by commercial producers for a particular species.

STEPS FOR COMPLETE DIET EVALUATION:

Setting the Baselines:

Ideally, all of the ingredients in a feed will have been fully evaluated using the Ingredient Evaluation. When evaluating an ingredient, ideally researchers would use a Control Feed that is Open Formula, so that the effect of each ingredient can be isolated.

Once an ingredient has been fully evaluated, feed formulators can ascertain whether the new ingredient can be included in the Complete Diet to evaluate the Complete Diet in its entirety. Ideally, the feed CD retains nutrient profiles (i.e. crude protein, fat) of the Control Feed.

In addition to the Complete Diet (the Test feed), the Feed evaluation should ideally select a Control Feed that is open formula, and a Reference Feed that is commercially available.

Compositional Analysis:

The first step of an evaluation is a compositional analysis of the Reference Feed, the Control Feed, and the Complete Diet. In all likelihood, there are probably multiple differences in nutrient values and ingredients among the feeds, so that although it can provide clues, one can not directly infer that the nutrient content is responsible for differences in fish performance. Often, manufacturers will provide feed tag values that are precalculated of ingredients and nutrient values. However, having a detailed set of independently "analyzed values" of the feed is particularly important if the data is submitted for publication. A list of recommended nutrient analyses to be conducted is found below. Most commercial analytical laboratories routinely conduct these tests;

-Crude protein
-Lipid
-Moisture
-Ash
-Fiber

-Minerals; Phosphorus, Ca, Mg, Na, Mn, Fe, Cu, Zn
-Amino acids; Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Tyr, Val, Taurine

Complete Diet Evaluation Workflow:

The diet evaluation should begin for the fish just after the larval stage, when grow-out diets are initiated. There are two types of studies:

1- Screening Study (laboratory scale). These studies screen the feeds, and make sure that the fish do not experience health or mortality issues.

If not successful, then improve the feeds.

If successful, then the next phase of Pilot Scale studies should begin:

2- Pilot Study

For both phases of testing, do not use surrogate species: focus on the target fish species of interest. Diets should be fed for the duration of the entire grow-out period.

Data Collection for each tank for both Screening Study and Production Study assessments:

Feed consumption,
Average initial weight, grams/fish
Average final weight, grams/fish
Starting and final number of fish
Water Quality, in flow-through and recirculating systems
Taste test?

Criteria to be used for both Screening Study and Production Study assessments:

Weight gain, percent of initial weight (WGI): The mean individual initial and final body weight of fish should be obtained dividing the total tank initial and final fish weight by the number of fish present in tank at start and end of study, respectively.

$$WGI = (Final\ fish\ weight - Initial\ fish\ weight)/Initial\ fish\ weight) * 100$$

Feed Conversion Ratio (FCR): It is the relationship between the feed fed to the fish and the weight gain of a population (pond, cage, pool, tank..etc). It is calculated as,

$$FCR = \frac{Kg\ of\ feed\ fed}{Kg\ of\ Weight\ gain}$$

FCR is a ratio and thus a unitless measurement. The lower the FCR, the more efficiently the fish metabolized the feed. It can be looked at as the amount of feed an animal requires to gain a kilogram of body weight. While FCR is a measurement of how efficiently a feed is utilized, it does not considered the differences in cost among different feeds.

Economic Feed Conversion Ratio (eFCR): A feed may be the more expensive but if weight gain and FCR are better, then the more expensive feed could be the most economical. The eFCR allows for this comparison.

$$eFCR = FCR * (\$/kg\ of\ feed)$$

**Please note that eFCR is one of the criteria looked at by sustainability measurement programs such as Seafood Watch:* total feed inputs divided by total harvested fish output over the entire production cycle. It should ideally be averaged over multiple production cycles and take account of seasonal differences (e.g., wet or dry season, age of fish).

Survival Rates (SR): Is the percentage of fish that grows to maturity in the tank. It is calculated as,

$$SR = \frac{Final\ harvested\ fish\ number}{Initial\ fish\ stock\ number} \times 100$$

1- Screening Studies

Screening studies should compare the performance of experimental feed (sustainable complete to the control feed (open-formula) and to a reference feed (closed-formula). Screening studies are best conducted at "laboratory scale" using multiple replicates (4 or more per diet is recommended) and multiple reference diets. Replication greatly reduces the economic risk of new feed evaluation and should build confidence when testing progresses to the more costly Pre-Production Studies. Please refer to the grow-out studies in Ingredient Evaluation for recommended protocols.[1]

How to determine pass/fail: The the weight gain, survival and eFCR of fish fed the experimental feed should meet or exceed that of fish fed the reference diet and the control diet. The average amount of gain (% of initial weight) observed during a trial

[1] https://f3fin.org/protocols/Grow-Out.pdf

should be at least 400%, and preferably more in order to conclude there is no difference among diets. If 400% gain is not observed, and there are no differences in performance parameters of fish fed the various feeds, the trial should be extended.

If pass - If the performance of the fish fed the new feed meets or exceeds the performance of fish fed the reference and control diets, the new feed should be tested in a pilot study.
If fail - If the performance of fish fed the new diet does not meet or exceed the fish fed the reference diets, it should be rejected until an improved formulation is available. An improved feed should be re-evaluated in a screening study.

Note that if an experimental feed performs equal to or better than a reference diet, a researcher can continue to grow these tanks of experimental and reference diet to market size out to determine survival, growth and FCR of the most promising experimental diet. This should be done with the 3 replicates (6 tanks total) in the field.

If the experimental feed still performs equal to or better than the reference diet, then the experimental feed can progress to the pilot stage, to be tested under field conditions with a larger scale as described in the next step.

2- Pilot Studies

The screening study, validates that the sustainable feed is nutritionally complete given specific growing conditions such as water temperature, dissolved oxygen, stocking rate etc. A pilot study needs to be conducted before the final step of facility wide adoption of a new feed,testing the new feed on a small portion of the tanks/raceways. The goal of the pilot study is to evaluate fish growth with the new feed under production conditions to reduce the economic risk of adoption. The duration of the study should span the entire grow-out phase.

Pilot studies for complete diet comparisons require on-site rearing conditions with the species of interest. Below descriptions are general guidelines. It is highly recommended that each farm facility works with an experienced fish nutritionist for optimal study design and result interpretation.

<u>Experimental set up requirements:</u>

- There should be sufficient replication (tank, cage, raceway) of dietary treatment groups. The replicate is considered to be the unit to which a feed is assigned.
- Fish density, feeding rates, and other environmental factors should mimic regular production.
- Researchers should record feed amounts accurately.

- Water quality should be measured in all replicates, with measurements for dissolved oxygen, ammonia, total suspended solids, and phosphorus as minimum parameters. Water quality monitoring should be conducted throughout the study.

How to determine pass/fail:

If pass: Feed is ready to be used throughout the farm, if it performs as well as or better than the existing feed as measured by eFCR.

If fail: Reject feed until an improved formulation is provided. If the growth parameters do not meet or exceed the control diet, the test diet is considered to have failed, and unable to perform on aquaculture farms in a commercial setting.

