The following protocol is in a process of continual improvement and will be updated periodically.
Last update: 08-25-2021

Grow Out

This protocol outlines the procedures that should be followed for grow-out studies in a laboratory scale setting. Grow out experiments evaluate the effect of ingredients on growth rates of fish. The results from grow-outs are critically important to farmers, who need to get to market as quickly as possible, and they are also important for FDA approval for a new ingredient in aquaculture feeds.

To assess the effect of ingredients on growth, we advocate as does Collins, et al (2012) that digestibility trials are conducted before growth trials, and encourage balancing all essential nutrients, including amino acids and phosphorus, on *digestible nutrients*.

Grow Out Design Considerations

a. Experimental Controls
- Check species partiality for floating versus sinking pellets.
- Select a commercial control feed that is the best-selling, so that it represents a good benchmark against the design feed.
- If possible, include a fishmeal control

b. Design feed (based on the ingredient of interest)
- Make sure that the design feed and all other feeds in the experiment are equivalent in all dimensions other than the ingredients:
    - all are either floating or sinking
    - all are made to the same pellet sizes and quantities so that they are administered corresponding to the growth stage of the animal
- Make sure that the design feed is comparable to experimental controls in terms of nutrition before manufacture:
    - Nutritional analysis should be done on ingredients and control feeds to inform formulation of the design feed
    - Formulas should be iso-nitrogenous and iso-lipidic to reduce variability; design feed matches the control.
    - Formulas should use ingredients of comparable quality: Review nutrient specifications of ingredients prior to formulating and manufacturing feeds. For instance, if a manufacturer sources high-quality fishmeal and low-quality poultry byproduct meal, then a comparison between the two will not be fair.

- Nutritional analysis should be done on the completed feeds as the feed manufacture process can change the nutritional composition, to determine if actual values differ from calculated values.
- In addition to evaluating the nutritional composition of the feeds, peroxide values may be measured before and after the feeding trial to give insight on potential rancidity to prevent or reveal a confounding variable.
- All feeds should be given expiration dates, and should be manufactured and administered before expiration dates of ingredients

b. Fish and culture

- Make sure that all tanks are of the same size and that there are enough tanks for 4 replicates per diet to better ensure experimental validity.
- Get eggs, hatch and grow on a commercial diet until at desired size (i.e. 4.8 g for Rainbow Trout). This diet should be different from the diets that will be fed during the trial.
- Assign diets randomly to each tank (based on the number of test and reference diets used). For rainbow trout, 20 individuals per 110 L tanks is recommended.
- Create a timeline for study that should allow for at least 400% expected growth depending on the age and size of the animal. For example, 12 weeks.
- Make sure tank sizes allow for 400% growth. Determine optimal stocking densities and environmental conditions for the species of interest.

c. Feeding protocol:

- The same feed protocol should be used for administration of all feeds. An example: feed fish by hand two times per day, 7 days per week to apparent satiation, which is achieved when the fish would no longer aggressively consume feed.

d. Experimental data collection:

- Take regular water quality measurements: temperature, dissolved oxygen, salinity, pH, alkalinity, ammonia.
- Take photographs pre-rigor at trial start and end to compare relative profiles
- Count fish and weigh as a group every 3 weeks. Individual weighing is only recommended at the beginning and end of the trial, as individual weighing can stress the animals and contribute to poor growth performance.
- Measure feed consumed weekly. This can be combined into 3-week periods so feed intake is expressed as a percentage of body weight at the end of the period.
- Euthanize ten fish from each tank at the end of the study for body composition analyses.
- Euthanize and freeze fish to compare fillet quality, taste and texture, and color. These observations can provide insight on how ingredients will impact the end product and consumer experience. Institutions such as Oregon State University perform formal sensory and consumer behavior research, however informal panels of opinion leaders may also be done to lessen cost. Note that fish will need to be purged prior to tasting if they are grown in a recirculating system.
- If growth issues are seen, conduct histological or hematological samples to diagnose issues.

- 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!

e. Chemical analyses

- Ingredients, diets, initial body composition (from stock population), final body composition (each tank), fatty acid profiles, and heavy metals contaminant information

f. Calculate performance indices

- Fish performance indices may be calculated using the following formulae. Other metrics may be used - these are most common.

Apparent feed conversion ratio (FCR) = feed intake (dry weight) / body weight gain (wet weight)
Hepatosomatic Index (HIS) = liver weight (g) / body weight (g) x 100
Feed intake (FI) = percent body weight per day
Gain (g) = final weight - initial weight
Percent gain = (Final fish weight - initial fish weight) / initial fish weight x 100
Carcass yield = (carcass weight / body weight) x 100
Fillet yield = (fillet weight / body weight) x 100
Viscero-somatic index (VSI) = (viscera weight / body weight) x 100
Gonadosomatic index (GSI) = (gonad weight / body weight) x 100
Condition (K) = [weight (g)/length^3 (cm)] x 100
Protein Retention Efficiency (PRE) = protein gained (g) / protein consumed (g)

g. Conduct statistical analysis

- Specify what analyses will be conducted to be consistent with experimental design
- Mitchell et al. (2017) suggest the following to obtain valid, useful results:
    - Reduce confounding by starting with comparable animals of the same size, age, species, etc. It is also important to utilize the same methods and procedures for all treatment groups (i.e., same feeding frequency, trial duration, water quality, tank conditions).
    - Develop a design with the number of individuals and treatment replicates necessary to have at least 80% statistical power. If this is not feasible, calculate the power of the design you are capable of running.
    - Determine thresholds to indicate significant difference in growth responses across treatments. Growth differences may be statistically significant, but are the differences large enough to warrant changing a fish farm's operations?
    - Consider which statistical analysis method is most appropriate for your study. Thorarensen et al. (2015) suggest that a mixed model ANOVA test is suitable for growth studies regardless of the fish species.

References:

Collins, S.A., Desai, A.R. Mansfield G.S., Hill, J.E., Van Kessel, Drew, M.D. (2012). The effect of increasing inclusion rates of soybean, pea and canola meals and their protein concentrates on the growth of rainbow trout: Concepts in diet formulation and experimental design for ingredient evaluation. *Aquaculture 344-349*; 90-99.

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Thorarensen, H., Kubiriza, G.K., Imsland, A.K. (2015) Experimental design and statistical analyses of fish growth studies. *Aquaculture, 448*, 483-490. https://doi.org/10.1016/j.aquaculture.2015.05.018