The follow protocol is in a process of continual improvement and will be updated periodically.
Last update: 08-01-2017

Grow-Out

Grow-out experiments evaluate the test ingredient in diets to determine its effect on fish health or target animal safety. The results from grow-outs are an important part of a FDA approval application package for a new ingredient in aquaculture feeds.

Formulation of the experimental diets is the most critical for comparing diets between laboratories. Collins et al. (2012) present a strong case for standardization, and for conducting digestibility trials before growout trials. Collins also argues that all diets should be balanced on a *digestible nutrient basis,* including all essential nutrients such as amino acids and phosphorus.

Most ingredient providers want to demonstrate efficacy of the ingredient in replacing fishmeal, since it is expensive. However, replacement can result in interactions between the pro-nutritional effects of fishmeal and the characteristics of the test ingredient (Collins et al., 2012). Because of these interactions, it is recommended that fishmeal content of the experimental diets be held constant or totally eliminated.

A first set of tests can be conducted in a laboratory setting using multiple replicated diets until a promising diet formula is developed for the test ingredient at pilot scale, followed by production scale trials using production conditions (i.e. water quality, stocking densities, feeding rates, etc.).

Sample Experimental design

a. Design Feed (Control diets and Test diets based on the ingredient)
b. Fish and culture – Get eggs, hatch and grow on commercial diet until at desired size (i.e. 4.8 g for Rainbow Trout)
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.
The study will last 12 weeks.
c. Feeding protocol: 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:
-Count Fish and weigh as a group every 3 weeks.
-Measure Feed consumed weekly. This can be combined into 3 week period 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.

-fillet quality,taste and texture

-histological or hematological samples if needed.

e. Chemical analyses; Ingredients, diets, initial body composition (from stock population), final body composition (each tank),

f. Calculation of performance indices

Fish performance indices were calculated using the following Formulae:

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 wt / (f – initial wt) / fish
Percent gain = (Final fish weight - initial fish weight) / initial fish weight x 100

G. Statistical analysis

Specify what analyses will be conducted to be consistent with experimental design

References:

Collins, S.A., Desai, A.R. Mansfield G.S., Hill, J.E., Van Kessel, Drew, M.D. 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. 2012.

