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

Digestibility

Digestibility

The determination of nutrient digestibility in specific ingredients and diets for fish has been an area of active research for decades. The Apparent Digestibility Coefficients (ADC) measure the percentage of nutrients in an ingredient that are available to the fish, Researchers, producers, and feed mills need ADCs to accurately formulate feeds and meet the needs of the animal without excess (Collins et al., 2012). ADCs are also needed to determine the nutritional and economic value of alternative ingredients. Data developed from many different laboratories have been compiled in publications such as the NRC (2011), and often show extreme variability. This is not unexpected since there are many factors that can affect the ADC of an ingredient, including basal diet formulation, method of feed manufacturing (cooking versus cold formation), fecal collection method, etc. Different laboratories often use a mixture of methods specific to that laboratory.

The nutritional value of an ingredient can be partially judged by determining the apparent digestibility of nutrients and energy and amino acid availability in compounded, extruded feeds. Nutrient and energy availability can be determined using the methods described by Cho et al. (1982) and Bureau et al. (1999) to estimate apparent digestibility coefficients described below (ADCs). Diets used for digestibility testing should include an theoretically indigestible (100%)marker such as laboratory grade yttrium oxide (recommended).

A complete control diet meeting or exceeding all known nutritional requirements should be used and blended with the test ingredients in a 70:30 ratio (dry-weight basis) to form test diets. The control diet below was formulated to be have good feed intake by trout, salmon and hybrid striped bass. The feed below includes squid meal but is also to low in phosphorus for the determination of phosphorus digestibility. This is because if the control diet contains high levels of phosphorous it is difficult to determine the phosphorus digestibility of the test ingredient. Phosphorus digestibility is important since it is a monitored nutrient in fish farm effluents by federal and many state regulatory agencies. This diet has been used successfully in several digestibility and growth trials with rainbow trout (Barrows et al., 2008, Gaylord et al., 2009, Barrows and Frost, 2014).

Recommended control diet:

Ingredient Composition		g kg l^{-1}
Squid meal [1]		250.0
Soy Protein Concentrate [2]		174.14
Corn Gluten Meal [3]		83.4
Soy Protein Meal [4]		43.0
Wheat Flour [5]		283.3
Tourine [6]		5.0
Menhaden Fish Meal [7]		133.9
Vitamin premix ARS 702 [8]		10.0
Choline Chloride [9]		6.0
Vitamin C [10]		2.0
Chromic oxide [6]		10.0
Ytrium oxide [6]	1.0	
Trace mineral premix [11]		1.0 .

[1] Wilbur-Ellis, Portland, Oregon, USA
[2] Solae Inc., Pro-fine VF, St Louis, Missouri, USA
[3] Cargill Inc., Minneapolis, Minnesota, USA
[4] Archer Daniels Midland Company, Decatur, Illinois, USA
[5] Nelsons and Sons Inc., Murray, Utah, USA
[6] Sigma Aldrich Company, St Louis, Missouri, USA
[7] Omega Protein Corp., Hammond, Louisiana, USA
[8] DSM Nutritional Products, Basel, Switzerland. Provides per kg diet before processing: Vitamin A 9650 IU, Vitamin D 6600 IU, Vitamin E 132 IU, Vitamin K 1.1 mg, thiamin mononitrate 9.1 mg, riboflavin 9.6 mg, pyridoxine hydrochloride 13.7 mg, pentothenate DL calcium 46.5 mg, cyancobalamin 0.03 mg, nicotinic acid 21.8 mg, biotin 0.34 mg, folic acid 2.5 mg, inostitol 600 mg
[9] NB Group Co. LTD, Shangdong, China
[10] Ascorbic acid Stay-C 35, DSM Nutritional Products, Basel, Switzerland
[11] Sigma Aldrich Company, St Louis, Missouri, USA. Contributed mg/kg of diet: zinc 30, manganese 13, iodine 6, copper 10, selenium 0.4

The quantity of an ingredient needed for this test is determined by the size of the extruder at the testing facility. For a typical laboratory size extruder (i.e. 44 mm screws) 6 kilograms is enough to make diets to complete both palatability and digestibility trials. The volumes suggested here are meant for referencing. The best practice would be to determine amounts of the ingredient with the facility preparing the feed.

Rainbow trout is often the species of choice for digestibility studies due to its resilience to stress and ease of handling for manual feces collection. Rainbow trout is considered a good surrogate for other species (i.e. salmon, yellowtail etc). Therefore, a large amount of data has been collected on trout through the years for ingredient performance comparison.

Apparent digestibility coefficients for each nutrient in the test diet and ingredients should be calculated according to the following equations (Kleiber 1961, Forster 1999):

$$ADCNdiet = 100\text{-}100 \ \{ \%\ Yt \text{ in diet } X \ \%\ \text{nutrient in feces} \} \ \{ \%\ Yt \text{ in feces } \ \%\ \text{nutrient in diet} \}$$

ADCNingredient = {(a+b) ADCNt -(a) ADCNr } b-1
where,
ADCNingredient = apparent digestibility coefficient of the nutrient in the test ingredient
ADCNt = apparent digestibility coefficients of the nutrient in the test diets
ADCNr = apparent digestibility coefficients of the nutrient in the control diet
a = (1-p) x nutrient content of the control diet
b = p x nutrient content of the test ingredient
p = proportion of test ingredient in the test diet

Diets should be manufactured by common commercial practice, or cooking extrusion. The processing conditions such as residence time in barrel temperature, moisture pressure should be recorded.

The experimental diets should be fed to 10-15 fish in each tank, weighing from 500 to 700-g each held in tanks of appropriate size (i.e. 500 L). Water temperature should be maintained near the physiological optimum temperature for each species (i.e. 15 ºC for rainbow trout). Each diet should be fed to a minimum of three tanks (4 or 5 tanks per diet is better) of fish.

Each diet, control and test, should be randomly assigned to a tank of fish and fed for at least two weeks before fecal collections begin. As opposed to Palatability testing, the first two weeks of the digestibility testing is done with separate diets (control and test) assigned to each tank. The diet assignment for tanks will stay the same throughout the experiment.

Fecal samples should be obtained by manual stripping when possible depending on species, ~16-18 h post-feeding. Manual stripping of all fish in each tank should be accomplished by netting and anesthetizing the fish, followed by gently drying and then applying pressure to the lower abdominal region to first express urine to a waste container and then fecal matter into a plastic weighing pan. Fecal samples for each tank should be freeze-dried and stored at -20 ◦C until chemical analyses are performed. Samples needed for analysis include, each test ingredients, each test and control diet, and feces from each tank. These samples will be analyzed for crude protein, lipid, energy, moisture, ash, amino acids and minerals including yttrium.

Adjustment of ADC values:

A variety of factors can result in negative ADC values or values above 100%. This most often occurs when the nutrient is present in very low levels in the test ingredient (i.e. fat in solvent extracted soybean meal) or has a true digestibility near to either threshold. In each of these cases the values have been rounded down to 100% or up to zero to assist in using these values for feed formulation.

For ingredients that are protein sources and have been through first feeding testing, palatability and digestibility testing can be conducted sequentially:

-2 weeks all tanks receive the bland control diet
-2 weeks test tanks receive test diet (70% control diet, 30% test ingredient) and feed intake measured- **Palatability**

-1 week feces collected on monday, wednesday and friday for rainbow trout.-
Digestibility

Caveats

The above procedures outline testing and measurement of an ingredient solely from a fish nutrition and physiology perspective. The procedures outlined above do not assess an ingredient using other measures of sustainability, or from an economic or business perspective. While an ingredient may pass from a scientific perspective, the cost of the ingredient to the feed manufacturer is equally important. For example, a high cost ingredient may yield positive results, but may not be economically feasible. These determinations are beyond the scope of the FIN effort.

References:

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