



The follow protocol is in a process of continual improvement and will be updated periodically.
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First Feeding Screening

First feeding trials (also referred to as Fry feeding trials) are designed to quickly screen a large number of potential ingredients to a more manageable number for further testing in digestibility, growth and other trials described here. These trials are less-expensive to conduct than grow-out trials and are highly recommended for ingredients that have not been tested before. Fry fish are an effective first look at ingredient effects since the fish at this stage of growth have a “naive” digestive system that has not been affected from previous diets.

If the test ingredient is a modification of an existing ingredients, such as an enhanced soy product, then this step can be bypassed.

The purpose of first feeding study is to investigate effects of the new ingredient on the fish growth and survival rates quickly and cost effectively. First-feeding studies cannot be considered substitutes for digestibility or grow out studies that obtain important qualitative and quantitative data on the performance of the new ingredients in the feed. Novel ingredients can be classified as either a protein or lipid/fatty acid source. First feeding studies are best for evaluating protein sources. Alternative lipid/fatty acid sources are best evaluated through a combination palatability, digestibility and grow-out studies. For protein source evaluation a fishmeal control diet that contains approximately 45% of a high quality fishmeal should be used. The test diets will then contain 5.0% of the high quality fishmeal and 40% of the novel protein. An example of feed formulations for a typical trial can be found in Table 1. To provide enough protein for this study, typically only 2 kilograms is needed, since very young fish do not need high volumes of feed.

Table 1. Ingredient composition (g/100 g) of experimental diets in fry screening trial.

Ingredient	control	Almond		Pistachio		Algae Co-P		Mussel meal
		Meal	Meal	Spirulina	1	2		
Fishmeal ^a	45.15	5.00	5.00	5.00	5.00	5.00	5.00	
Wheat gluten meal ^b	6.50	6.50	6.50	6.50	6.50	6.50	6.50	
Corn protein Conc. ^c	2.80	2.80	2.80	2.80	2.80	2.80	2.80	
Almond meal ^d	0.00	58.00	0.00	0.00	0.00	0.00	0.00	
Pistachio meal ^e	0.00	0.00	51.40	0.00	0.00	0.00	0.00	
Spirulina ^f	0.00	0.00	0.00	46.23	0.00	0.00	0.00	
LEA strain 1 ^g	0.00	0.00	0.00	0.00	54.96	0.00	0.00	

LEA strain ^h	0.00	0.00	0.00	0.00	0.00	54.76	0.00
Mussel meal ⁱ	0.00	0.00	0.00	0.00	0.00	0.00	46.50
Wheat flour ^j	29.35	2.34	11.21	15.94	7.05	10.04	16.36
Fish oil ^k	11.40	11.50	10.20	11.61	13.50	10.70	10.80
Lysine HCl	0.00	2.55	1.65	1.98	1.40	1.40	1.75
Methionine	0.00	0.80	0.55	0.40	0.28	0.38	0.75
Threonine	0.00	0.57	0.60	0.00	0.10	0.10	0.00
Dicalcium phosphate	0.50	4.25	4.40	3.85	2.60	2.63	3.85
Lecithin	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Vitamin premix ^l	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Trace min premix ^m	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin C ⁿ	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Taurine ^o	0.00	0.50	0.50	0.50	0.50	0.50	0.50
Potassium Chloride	0.00	0.56	0.56	0.56	0.56	0.56	0.56
Sodium Chloride	0.00	0.28	0.28	0.28	0.28	0.28	0.28
Magnesium oxide	0.00	0.05	0.05	0.05	0.05	0.05	0.05
Crude Protein, % dmb	46.5	47.9	46.8	47.7	46.9	45.0	46.04
Lipid, % dmb	19.9	20.4	19.7	18.7	18.7	20.7	19.9
Moisture, %	4.8	5.1	5.4	5.6	7.3	4.9	5.8

We recommend rainbow trout as a good surrogate species since their eggs are abundant and the first feeding fish typically have high survival rates when fed a fishmeal based diet. After the eggs hatch, and the fry “swim-up” to the surface of the tank, and are ready for the experimental tanks. A typical situation involves placing 100 swim-up stage trout in 110 liter aluminum troughs supplied with 4 L min⁻¹ of partial reuse water maintained at 15°C. All diets (test and control) are fed to a minimum of 4 tanks of fish and fed to excess so that the fish are satiated using belt feeders 7 days per week for 6 weeks. Fish should be weighed as a group and counted after 3 weeks of feeding. Then, they should be counted again at termination of the study at 6 weeks.

Percentage of the fish surviving, and weight gain should be calculated for each tank. Any ingredient that significantly decreases survival, when compared to the fishmeal control diet, is considered to “fail” and would not move to next stage. If an ingredient fails, the ingredient providers should reevaluate processing methods and analyze whether processing methods can ameliorate anti-nutrients or contamination. If survival and growth of the trout fed novel ingredient equals the control diet, then this ingredient is considered to have “pass(ed)” and moves on to functionality, palatability and digestibility testing. If there is a slight reduction in growth rate of trout fed a novel ingredient, the evaluation process should continue. The palatability and digestibility trials will determine if the slight growth issues are due to a lack of feed intake or low nutrient digestibility.