The follow protocol is in a process of continual improvement and will be updated periodically.
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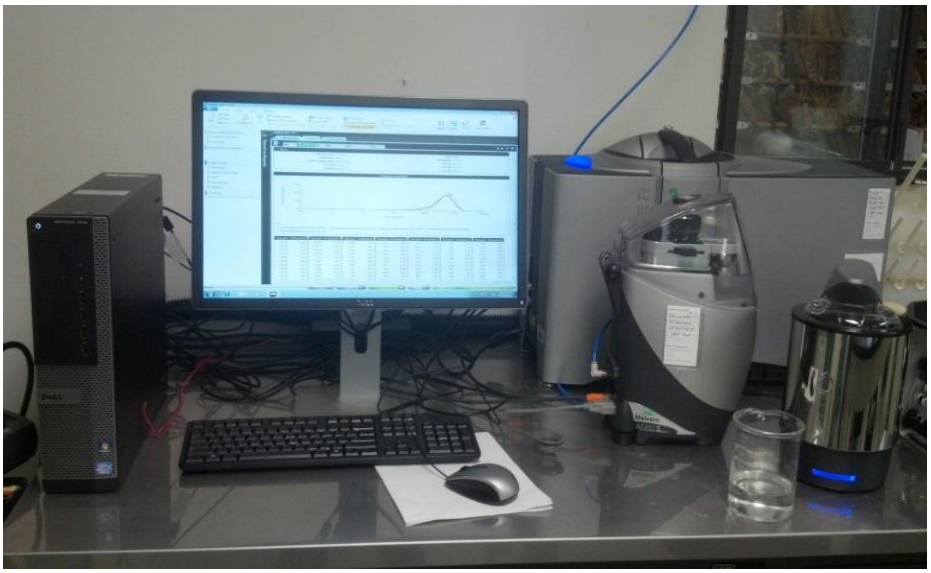
Solid Waste Management

Solid waste management is a topic of great concern in any type of animal agricultural whether terrestrial or aquatic for both environmental and production reasons. In aquaculture production the diet formulation will affect both the levels of dissolved nutrients such as nitrogen and phosphorus, and the quantity and consistency of the solid waste. Fish rearing systems employ a variety of different methods to remove solid waste (uneaten feed and feces) from the water and these can include settling and filtration. Some ingredients can cause a diarrhea like effect resulting in fine and suspended feces. This results in poor water quality which adversely affects fish health and can be avoided through proper feed formulation and feed production methods. For recirculating aquaculture systems (RAS) and flow through systems heavy, durable feces that settle or filter out easily is needed. Net pen production may benefit from easily dissolving feces to prevent benthic accumulation. All this can be a function of inclusion levels of the ingredients in the feed with trout fed plant-based diets (fishmeal free) a good example.

To improve the sustainability of aquaculture very technological methods have been developed to study the stability and rheology of fish feces when fed plant based diets (Brinker and Friedrich, 2012). This method uses plate on plate rheometer running in different flow modes (creep and oscillation) to evaluate the feces of fish fed different diets.

Fecal particle size is considered a direct measure of the mechanical stability of feces in self-cleaning tank systems (Brinker, 2007). Another approach to evaluate the fecal particle size and durability involves the use of a radial flow separator (RFA) on the effluent of each test tanks (Figure X in “protocol photos” file). All effluent leaving the experimental tanks flow through the RFS which accumulates all solids in the bottom of the cone. The experimental feeds are fed for two weeks during the day. After two weeks at the end of the day, the tanks and RFS are cleaned of all waste and uneaten feed. The next morning feces can be collected from each RFS by opening the valve at the bottom and collecting the liquid and solids into a pan.

This slurry is then analyzed by a laser refraction methodology to give a complete quantitative size distribution (Mastersizer 3000, Malvern Instruments Inc., Westborough, MA).



In this system the fecal slurry is placed in a reservoir connected to the instrument. When activate a small propeller in the analyzer suspend the feces throughout the water column. The suspension is then sent to chamber where the diffraction of 22 lasers is measured to determine the size and distribution of the particles. This equipment is often used in the pharmaceutical and food industries. The instrument is set to take 25 measurements. And during that time the feces are subjected to agitation by the propeller which reduces particle size. The first measurement is considered an estimate of the true particle size. The difference in size between the first and 25th measurement is considered an estimate of durability. Both of these measurements have been shown to be affected by diet and feed manufacturing method.

The number of fish in each tank will need to be determined based upon (i) feed consumption of the fish,(ii) dry matter digestibility of the diet and (iii) the capacity of the analyzer. This is because the accuracy of laser diffraction instrument can be compromised by a slurry that is too “thick”. The instrument measures the obscuration rate (amount of laser light blocked in test chamber) and if it is above X then the number of fish or the amount of feed offered needs to be reduced.

References:

Brinker, A., Guar gum in rainbow trout (*Oncorhynchus mykiss*) feed: the influence in quality and dose on stabilization of faecal solids, Aquaculture 267:315-327. 2007.

Brinker, A., Friedrich, C. Fish meal replacement by plant protein substitution and guar gum addition in trout feed. Part II: Effect on faeces stability and rheology. Biorehology 49:27-48. 2012.

