Northeastern Regional Aquaculture Center

Evaluation of Artificial Diets for Cultured Fish

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Introduction

One of the major expenses in any fish culture operation is the cost of feeds for the fish, and the profitability of many operations is frequently tied to the cost of feed. Hatchery production of fish larvae most often requires the expensive production of live food (phytoplankton and zooplankton), because artificial diets are either not available, or are grossly inadequate. Artificial diets are available for growout of fingerlings and adults of most cultured fish species, but they may be less than optimal because they had been formulated for another species. For example, in the United States,commercially formulated diets are available for catfish and salmonids, but these diets have been used without modification to feed other species of fish, including hybrid striped bass, tilapia, carps, and others. Less than optimum diets for growout of fingerlings will result in lowered growth rates and excessive waste, either by excessive fecal material, excessive urinary nitrogen, or uneaten food. Thus, less than optimum diets are not only wasteful in terms of money spent on feed, but they can cause increased waste management problems. The key challenge of producing production feeds is the maximization of fish growth with a minimization of waste.

The challenges of formulating diets for larval fish are more formidable, as evidenced by a reliance on live feeds. One major challenge of larviculture is the production of organisms that areas similar as possible to those in nature. Marine fish larvae that hatch and grow in nature typically feed on zooplankton in an environment of low fish density and good water quality; mortality is often >90 percent, due primarily to problems at first feeding and to predation. By contrast, those that hatch and grow in larval rearing systems typically feed on rotifers, *Artemia* and prepared diets in an environment of high fish density and (at best) adequate water quality; mortality, due primarily to

problems at first feeding and disease, can often be greater than 50 precent. The development of high-quality artificial diets could potentially ameliorate water quality and disease problems, as well as reduce the high cost of live feed.

Evaluation of Production Diets

For most practical applications, evaluation of production diets (diets for fingerling and adult production) can be adequately done in feeding trials. Since diets are available that have a well-defined composition, growth performance of fish can be readily determined after modifications of a control diet are made. Typically, the total feed utilization by fish, expressed as food conversion ratio (FCR), or the protein utilization, expressed as protein efficiency ratio (PER), are calculated. The highest quality production diets will have relatively low FCRs and high PERs.

One of the simplest means for an aquaculture producer to assess feed performance is to determine a food conversion ratio (FCR). The FCR is the weight of food supplied divided by the weight gain of the fish during the feeding period. FCR can be expressed by the equation:

(1)
$$FCR = F/(W_f - W_o)$$

when, F is the weight of food sup plied to fish during the study period,

         W_o is the live weight of fish at the beginning of the study period,

and W_f is the live weight of fish at the end of the study period.

Example: A fish pond operator starts with 1,000 fingerlings at an average weight of 200g each. The aggregate W_0 is 200 kg. The fish are fed 7g food/fish/day for 6 months when they are harvested at a final average weight of 900g each, but there has been 2 percent mortality. The aggregate W_f would be:

1,000- (1,000 x 0.02)= 980 fish x 900g = 882kg

The amount of food supplied would be:

7g/day/fish x 182 days x 1,000 fish= 1,274kg

Then, the FCR would be:

FCR = 1274kg/(882kg - 200kg) = 1.87

A very important factor to remember when FCRs are compared is that they are based on the *wet* weight of the feed. Different feeds may have very different moisture levels. For example, a dry catfish production diet may have a moisture content of around 10 percent, whereas a semi- moist diet for sea bass may have a moisture content of over 60 percent. Moisture does not contribute to the growth of fish, but does add a bias to the FCR values. Thus, if comparisons are made between two or more diets, it is often useful to calculate the FCR on a *dry* weight basis. To make this easier, it is important to know the percent of moisture and dry weight in both your feeds and fish.

High protein ingredients are frequently the most expensive components of artificial diets. Consequently, feeding a diet too high in crude protein will not only be wasteful in terms of cost, but excess excretory nitrogen resulting from the breakdown of protein for energy metabolism may be a stressor to the fish. One means for determining the optimum level of protein in a selected feed is to compare the protein efficiency ratios (PER) of different feeds fed to fish. PER is the weight gain of fish divided by the dry weight of protein in the feed. An equation describing PER would be:

(2) $$PER = (W_f - W_0)/F \times p$$

when, F is the weight of feed supplied over the test period,

and p is the fraction of crude protein weight in the feed,

For example, if the percentage of crude protein in the feed from the above example were 40 percent, the PER over the 6 month growth period would be:

PER = (882kg - 200kg)/(1,274kg x 0.4) = 1.34

Now, if a feed were chosen with the reduced crude protein content of 35 percent and the fish growth is the same, the PER would be:

PER = (882kg - 200kg)/(1,274kg x 0.35) = 1.53

The PER values are reduced when protein levels in the feed are either insuffient or are in excess. Optimum protein content in fish feeds is species specific and occurs when PER is maximized.

Evaluation of Larval Diets

Diets for larval fish are notoriously difficult to evaluate because there are no completely defined artificial diets that are adequate for fish growth. Larval fish producers are currently reliant upon live feeds, so active work with artificial diets is largely confined to the research community. A comprehensive evaluation of an artificial diet only begins with a well-controlled experiment to compare survival, growth, and perhaps other indicators (e.g., stress/activity tests) of the larvae to those obtained with live food (either rotifers, *Artemia* or natural zooplankton). If equivalency is not obtained, one then needs to investigate the causes of the deficiency, realizing that those causes may not even be in the formulation of the diet. Two basic categories of research are required: 1) research on the physical and chemical state of the diet in the water column; 2) research on the physiological and biological requirements of the larvae. Two special caveats should be noted here: 1) all research should be conducted, and the results expressed, relative to live food; and 2) if the artificial diet happens to be microncapsulated, it is necessary to investigate deficiencies in the diet and the microcapsule separately. One way to study the microcapsule separately is to microencapsulate live food, as Leibovitz (1991) has done with *Artemia* nauplii.

Diets in the Water Column

After they are introduced into the water column, diets should remain both available and palatable to the larvae without leaching significant amounts of nutrients. Ideally, the diet should be neutrally buoyant; but in practice, this is very difficult to achieve. Many sinking diets can be kept in the water column with sufficient aeration, but the aeration levels required may be detrimental to the larvae due to the excessive agitation. Estimation of the availability of the diet to the larvae is possible even without the larvae in the system simply by measurement of the residence time of the diet: 1) at the surface; 2) in the water column; and 3) at the bottom. For example, Leibovitz (1991) quantified the percentages of diet particles (microencapsulated *Artemia* nauplii) at each of the three locations over an 8-hour period to demonstrate that they spent 2-4 hours

at the surface, 1-2 hours in the water column, and the remainder at the bottom.

Leaching of essential nutrients from larval diets has long been considered a serious problem, with water soluble vitamins being the most susceptible (Meyers 1979). Microencapsulated and microbound diets can help to overcome leaching, but the diets should still be analyzed to determine the extent of the problem in a comprehensive examination scheme. The degree of leaching can be determined by chemical analyses for various substances conducted either on the diet particles recovered from the water at various time intervals, or on the water itself, or on both. For example, Leibovitz (1991) showed that microencapsulated *Arternia* exhibited no significant change in proximate composition after 2 hours in seawater. The simplest determination of leaching includes simply the measurement of dry weight of particles recovered from the water at various time intervals.

Palatability can be determined by the rate of the rate of ingestion of feed particles by larvae. Simple visual observations can be useful, provided that the larvae and particles are large enough to be seen with the naked eye. Alternatively, larvae can be videotaped to record the number of strikes at prey (or particles), number of successful ingestions, and number of rejections. The recent use of image analysis to determine number of prey remaining in a bowl with predator(s) at frequent time intervals (Letcher 1990) could be adapted for palatability determinations, but has so far been attempted only with live *Artemia* as prey.

Determining Digestive Capabilities and Nutritional Requirements of Larvae

The digestive capabilities and dietary requirements of the larvae can best be determined through a combination of biochemical, physiological, and morphological studies. Perhaps the greatest challenge to larval fish nutritionists is the integration of information from those studies in the formulation of adequate artificial diets. Some approaches to estimation of the nutritional requirements of a given species have included biochemical analyses of: 1) yolk material in eggs of that species; 2) zooplankton on which the species feeds, or 3) *Artemia* (Leibovitz et al. 1987). Estimation of the physiological capabilities of larvae have included studies of the development of digestive enzyme production (e.g., Baragi and Level 1986) and determinations of the pH of the digestive tract in which the enzymes must function (e.g., Buddington 1985). Of particular value are those studies that locate the portions of the digestive tract responsible for the addition of specific enzymes through histochemical means (e.g., Segner et al. 1989). The relative roles of exogenous and endog-

enous enzymes in the digestion of live vs. artificial food in the larval fish digestive tract has been studied and debated for years (Dabrowski and Glogowski 1977a, 1977b); however, the addition of digestive enzymes to artificial diets has had varying degrees of success/failure (Dabrowski and Glogowski 1977c; Dabrowska et al. 1979; Lauff and Hofer 1984; Tandler and Kolkovsky 1991).

Morphological studies of development of larvae include histological and histochemical methods with light, scanning electron and transmission electron microscopy. In the context of determination of larval capabilities for utilization of artificial diets, the most useful studies include examination of sensory apparatus (e.g., taste buds) (Appelbaum et al. 1983), the alimentary canal (especially the mucosal epitheliums) (e.g., Kjorsvik et al. 1991; Verreth et al. 1992), the liver and pancreas (Alami-Durante 1990). Larval fish are characterized by significant uptake of nutrients by the hindgut epithelial cells and intracellular digestion in the supranuclear vacuoles of those cells (Iwai and Tanaka 1968; Watanabe 1984). Any morphological examination of fish larvae by researchers should emphasize the development of hindgut epitheliums.

Once the diet is ingested, the digestion, absorption, and assimilation of the food can be studied using fluorescence, radiolabeling, and/or histological methods. Walford et al. (1991) and Walsh et al. (1987) have used fluorescence methods to follow particles passing through the larval fish digestive tract, particularly noting time of passage and bottlenecks to passage. Determination of assimilation efficiency with radiolabeled carbon has long been practiced with larval fish fed live food (e.g., Boehlert and Yoklavich 1984), but has recently been used also to compare uptake of artificial and live diets (Tandler and Kolkovsky 1991). Assimilation effiency data, when combined with data on rates of ingestion of live vs. artificial diets, can provide valuable insight into artificial diet deficiencies (e.g., to what extent reduced growth is due to reduced ingestion vs. reduced digestibility). Whereas fluorescence and radiolabeling studies are most useful in estimating process rates for the whole organism, histological studies are most useful in identifying specific digestion and absorption sites within the digestive tract. Bengtson (1993) and Bengtson et al. (1993) have studied uptake of live vs. artificial food by examination of mucosal epitheliums in larval fish. By sampling larvae at time intervals after a single feeding and examining histological sections, one can follow the passage of particles through the digestive tract and answer the question: Are there differences in the digestion and absorption of live vs. artificial diets? Larval striped bass appear to absorb all of the nutrients

from live *Artemia* nauplii through the hindgut epithelial cells, but do not absorb nutrients from artificial diets through those cells.

Conclusion

The evaluation of artificial diets for adult and juvenile fish has been largely based upon feeding trials with great success, because defined basal diets are available. Aquaculture producers can use simple feeding trial techniques to evaluate the efficiency of feed utilization by their fish and the cost-effectiveness of feeds from different sources. Evaluating feeds for larval fish is not as simple because of the lack of an adequate artificial basal diet. Research has evolved from the one-dimensional approach of formulating a variety of diets and simply obtaining survival and growth results from feeding trials with larval fish. The multi-disciplinary nature of a comprehensive evaluation of an artificial diet now requires that many groups communicate and cooperate with each other. These groups include, but are not limited to, nutritional biochemists, food chemists and engineers, physiologists and morphologists, and the Aquaculturists themselves. Continuation (and undoubtedly expansion) of such a multidisciplinary approach provides the best chance of defining an inert or artificial diet that can compete with live rotifers and *Artemia* as a nutritional source for marine fish larvae. The ideal artificial diet, however, will produce marine fish larvae that biochemically, physiologically, and behaviorally resemble wild larvae that feed on natural zooplankton.

Acknowledgments

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