

**WINTER SCHOOL**  
**ON**  
**Recent Advances in**  
**Mariculture Genetics**  
**and Biotechnology**

**4<sup>th</sup> to 24<sup>th</sup> November 2003**

**Course Manual**



**Organizing committee:**

**Prof (Dr.) Mohan Joseph Modayil**  
**Director, CMFRI, Kochi**

**Dr. P.C. Thomas**  
**Winter School Director**

**Co-ordinators:**

**Dr. R. Paul Raj, Head, PNPB**  
**Dr. K.C. George, Principal Scientist**  
**Dr. P. Jayasankar, Senior Scientist**  
**Dr. D. Noble, Senior Scientist**

**INDIAN COUNCIL OF AGRICULTURAL RESEARCH**  
**CENTRAL MARINE FISHERIES RESEARCH INSTITUTE**  
P.B. No. 1603, Tatapuram P.O.,  
Kochi – 682 014

## **AQUAFEED BIOTECHNOLOGY-AN OVERVIEW**

**R. Paul Raj**

*Central Marine Fisheries Research Institute, Cochin.*

### **Introduction**

Aquafarming is emerging as a successful small-scale bio-industry in India. In order to sustain this industry an organized feed industry is essential to manufacture and supply quality controlled compounded feeds for the different stages and production systems. The exogenous feeds used in aquaculture can be broadly grouped into (i) supplementary feeds and (ii) complete feeds. Supplementary feeds, by and large are empirical formulations, which do not provide the essential nutrients in adequate levels and balanced proportions as in the case of complete feeds. Supplementary feeds can be either made of a single feed ingredient or a mixture of ingredients; but usually low-cost agricultural and animal wastes, and by-products are used. Thus, the production achievable through supplementary feeding depends on the quality and quantity of the supplementary feed and on the natural food produced in the ponds.

In the semi-intensive and intensive systems of fish and crustacean culture provision of nutritionally balanced complete feeds is essential to achieve maximum production. In these systems feed forms the major operational input and often accounts for 40 to 60 per cent expenditure. Therefore, it is of foremost importance that the feeds are scientifically formulated to provide all the essential nutrients and energy in adequate levels and balanced proportions, optimally processed and judiciously supplied to achieve maximum efficiency at optimal cost.

### **Need for ecofriendly feeds**

Feed holds the key not only for successful aquaculture but also for its sustainability. For instance, until the late 1980s choice of shrimp feed was primarily based on its cost, potential FCR, growth and production potential. Concerns of environmental degradation, disease outbreaks, mortalities, and poor growth and production in the 1990s have posed new challenges to aquafeed industry and aquaculture. Wastes derived from unconsumed food, faeces and metabolism of nutrients are the major contributors to pollution in poorly managed farms. These wastes are excellent nutrient media for the propagation of microbes, some of which are pathogenic while others like luminescent bacteria produce toxins which induce stress, and predispose the shrimp to diseases, cause reduced growth and production from farms. Excessive waste nutrients in turn cause plankton blooms, drastically alter the dissolved oxygen profile in shrimp farms due to high BOD and COD, and produce toxic gases like H<sub>2</sub>S. Waste production is related to the performance of a feed in culture and feed conversion ratio (FCR) could be used as an index of the feed based wastes (Table 1). A rough estimate of the waste production from shrimp culture is given in Table 2. In a

four-month crop about 47 % of the organic matter, nitrogen and phosphorus wastes are generated during the 4<sup>th</sup> month of feeding. Thus it is imperative to produce less pollutive, quality assured feeds and evolve eco-friendly feed management strategies for culture.

### **Characteristics of eco-friendly feeds**

Feed quality is defined by the physico-chemical characteristics matching the specific requirements of the grow-out stages at optimum intake, with minimum output of feed, faecal and metabolic wastes. The factors, which determine the quality of a feed are its nutrients profile, anti-nutrient status, particle size, texture, stability of nutrients, attractants, digestibility, analytical accuracy and shelf life. The production of nutritionally adequate feeds require selection, quality control and biological evaluation. A well balanced diet not only results in higher production but also provides the nutrients necessary to hasten recovery from diseases or aid the animal in overcoming the effects of stress. Design and fabrication of complete artificial diets need information on a variety of biological, physiological, biochemical and nutritional aspects, besides the intensity of culture operations. Information on the dietary levels of essential nutrients that promote maximum growth at optimum intake, the preferred form of diet, feeding behaviour, role of attractants, feeding stimulants and deterrents, digestive capabilities of the species and also about the feed ingredients and additives that should go onto the feed for proper ingestion and utilization by the animals is required.

### **Balance of nutrients**

A balanced feed formula should include an energy source plus sufficient indispensable amino acids, essential fatty acids, phospholipids, specific vitamins and minerals to sustain life and promote growth. In the case of crustaceans, a source of sterol is also necessary. One of the major constraints in developing high performance eco-friendly feeds is the non-availability of complete data on quantitative nutrient requirements for the different stages.

The nutritive value of a dietary protein is dependent on its amino acid profile and their bioavailability. All the essential amino acids, viz., arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine should be found in adequate levels and balanced proportions. Amino acid imbalance besides affecting growth and feed efficiency, leads to increased production of nitrogenous wastes mainly in the form of ammonia. Similarly excess of protein in the diet, more than necessary to sustain protein synthesis, also results in nitrogenous waste production.

Nitrogen is the major source of waste derived from protein in the food. Reduction in nitrogen output could be achieved by optimum use of amino acid-balanced protein, by maintaining optimum protein-energy ratio and by ensuring balance of non-protein nutrients. Amino acid imbalance in the diets can also occur during processing of feed proteins and manufacture of feeds. Excessive heat treatment markedly reduces protein digestibility and biological value due to the destruction of amino acids by oxidation through the formation of linkages between individual amino acids, which are more

resistant to digestion. Bioavailability of lysine and methionine are markedly affected by improper processing. Heat treated fish meals containing free histidine and histamine have decreased EAA availability. There are also reports of antagonism between amino acids when they are found in disproportionate levels. Leucine/isoleucine antagonism as well as arginine/lysine antagonism are well established. Even when a protein has well balanced amino acid profile, its utilization can be affected if protease inhibitors are present in the ingredients used.

It is therefore necessary to prepare a protein-mix, which satisfies the amino acid needs of the species. The levels of digestible energy, essential fatty acids, vitamins, and anti-metabolites also significantly influence the protein utilisation and nitrogen waste output.

Crude fibre levels should be carefully monitored and should be maintained within optimal levels. High fibre levels offer low food retention time in the digestive tract for the enzymes to effectively digest the ingested food. Thus a good proportion of the food is excreted as faeces, in high fibre diets.

Quantitative requirements of most of the vitamins have not been clearly defined for shrimps. Vitamin premixes currently in use are mostly empirical formulations. Water soluble vitamins as a group need monitoring as some of the vitamins leached from the diet, and unconsumed feed promote the growth of phytoplankton and microbes, occasionally causing blooms. Thiamine, biotin, and cyanocobalamin have been known to trigger blooms.

Mineral and trace element requirement of shrimps are also not well established. Phosphorus is considered to be the dominant element in the feed derived wastes being about 86 % in shrimp culture. Dietary source and level of phosphorus affect its utilisation by shrimps. Phosphorus from plant ingredients and tricalcium phosphate are poorly utilised. Eco-friendly feeds should contain optimum level (0.9%) of bioavailable phosphorus. Excess of trace elements such as Zn, Co, Fe, Mn in the diets, when excreted also trigger phytoplankton blooms as they are essential for growth and multiplication of phytoplankton.

#### **Anti-Nutrients**

Several anti-nutrients (toxic principles) occur in some of the raw materials used in feed formulation (Table 4). Gossypol, Cyclopropenoic acids, thioglucosides, cynogens, hemagglutinin, phytic acid, mycotoxins, antivitamin, antimineral and antienzyme inhibitors fall under this category. Inclusion of ingredients containing any of the toxic principles could affect the food intake, bioavailability of nutrients and also nutrient metabolism and cause physiological and metabolic disorders. Such ingredients should either be excluded from the diet or added after adequate preprocessing to remove the toxic principles, thereby minimizing wastage of feed.

## **Additives**

Binders, antioxidants, antimicrobial agents, attractants, feeding stimulants, growth promoters, pigments and therapeutants are essential in feed to meet specific needs. Judicious use of these additives either directly or indirectly helps to reduce feed derived wastes in shrimp culture. However, therapeutants, especially antibiotics should be used with utmost care adhering to prescribed dosage and schedules. Immuno-modulators such as  $\beta$ -glucans, lecithins, lipopolysachrides and other bioadditives, which have been shown to enhance disease resistance should be used in disease management programmes.

## **Quality Of Raw Materials**

The quality of a feed is greatly determined by ingredient. Ingredients with high levels of non-protein nitrogen, crude fibre, ash, salt content, oxidized lipids and anti-nutritional factors should be excluded from shrimp feeds.

## **Processing**

Microgrinding and homogenous mixing of ingredients, premixes and additives, optimum conditions of pelleting, cooling and drying are very important for proper utilisation of the feed by shrimps and to minimize feed waste. Microgrinding makes available more surface area for enzymatic action and hence improves digestibility of the feed. Fine grinding also helps in better gelatinization of starch and binding. Mixing processes should ensure uniform dispersion of the macronutrients and additives so that each unit weight of the finished feed has a balanced nutrient profile.

Fish feeds may be pelleted by what is sometimes called the "extrusion" process, thereby expanding rather than compressing the various ingredients. Variations in formulation and processing allow a wide range in bulk density; 0.25 - 0.3 g/cc being common for fish feeding. This feature makes them attractive for certain types of fish culture. Fish may be observed while eating and the amount of feed limited to that which is accepted. The number of fish in a water impoundment and their health can be observed without sampling. In general, the processing of expansion of extrusion consists of: (a) conditioning soft feed which is in meal form to contain 25-30 percent water, (b) conveying this conditioned feed by auger into a pressure-sealed cylinder, (c) injecting steam, thus decreasing friction of material within the cylinder and increasing gelatinization of raw starch, (d) extruding to atmospheric pressure, almost exploding the material through holes in the die plate at the end of the cylinder, (e) cutting off the extruding ribbon at the outside of the die plate by a rotating knife, and (f) drying the pellets in a high-temperature oven at about 120 C to a moisture content suitable for storage.

Several details should be added to this general process description for a better understanding of floating pellets. The feed formula is important in obtaining a desired

expanding texture. Cereal grains can be expanded to a very low bulk density, whereas protein concentrates low in starch may remain unaltered in bulk density. Raw starch is a requirement of a good floating pellet; 90 percent being gelatinized during the 30 to 60 seconds the feed is in the expander cylinder. Pressure builds up to several atmospheres due to forward passage of the material into a smaller volume. Superimposed steam causes a high product temperature, changing the consistency of the material from a free-flowing meal to a dough. By the sudden release of pressure at the discharge end, the feed assumes a "puffed rice" texture like some breakfast foods and snacks.

Following oven drying, a standard pellet cooler is often used to lower product temperature after internal moisture is less than 13 percent. Even with this treatment, previous high temperatures partially destroy heat-labile vitamins and decrease the availability of some amino acids. Rather than over-fortifying the formula before pelleting, as is done for preparation of hard pellets, heat-sensitive additives may be sprayed onto expanded pellets after extrusion.

Biological tests under pond conditions with natural food available have shown no difference in growth of catfish and goldfish using a formula feed processed by hard pelleting and by the extrusion process. Also no significant difference was found chemically in total crude protein or in individual amino acids for several formula feeds each prepared as hard pellets and as extruded pellets. However, testing fish growth in tanks and cages has revealed a partial destruction of some nutrients in extruded feeds.

There is evidence that fish fed with floating pellets contain more liver and body fat than those fed the same formula feed processed as a hard pellet. This may be due to the increased digestibility of the carbohydrate part of the ration. The expansion process is expensive compared with other methods of feed manufacture in terms of equipment cost, heat used in generating steam and in oven-drying, and in loss of nutrients. It may be concluded that the value of extruded pellets is best measured by practical feeding tests using data on feed conversion, cost of management, and acceptability by fish as deciding factors.

### **Feed Particle Size**

Feed particle size is very important for proper utilisation. Particles should be adequately sized to suit the mouth parts and digestive capabilities of the animals. Under-sized and oversized feed particles should be screened before feeding to prevent feed wastage, degradation of the culture environment, and propagation of pathogenic organisms.

### **Digestibility**

Shrimps have a very small digestive tract, which is capable of utilizing soft food material, similar to that of live food, which they consume in nature. Artificial dry diets should have rehydration characteristics and render flexibility for feeding by the shrimps. Addition of exogenous enzyme or inclusion of enzyme or bio-preprocessed ingredients would further improve the digestibility of nutrients.

## Shelf-life

Prolonged storage lowers the biological value of feeds. Shelf-life is also dependent on the shelf-life of ingredients used in the feed. Ingredients and finished feeds should be used as fresh as possible. Prolonged storage alters the quality due to enzymatic action and chemical changes. Chemical changes included breakdown of lipids, formation of free fatty acids and rancidity. Rancidity reduces the palatability of the feed and the availability of amino acids, and certain vitamins. Raw material and compounded feeds being highly nutritious attract insects such as beetles, weevils and moths, which expose the feed to fungi and introduce contaminating bacteria. Fungi produce highly potent mycotoxins like aflatoxins, which are toxic to shrimps. Vitamin potency decreases during storage. Feed stored for periods exceeding six months need thorough chemical and biological evaluation for acceptability and digestibility.

## Eco-Friendly Feed Management

Judicious feed management is also an important factor in achieving good feed efficiency and reducing feed wastage. Waste production could be reduced by selecting feeds which are freshly made, quality assured, proven with best potential FCR, rejecting feeds which have lost their nutritional potency and have poor acceptability to shrimp and poor stability when introduced into water, by using feed of appropriate particle size designed for a particular stage, and by regulating the ration and feeding schedules with reference to feeding guides, response of the animals to the feed and environmental conditions.

Table 1: Relationship of FCR to waste production per tonne of shrimp

FCR	Organic Matter (Kg)	Nitrogen (Kg)	Phosphorus (Kg)
1.0	500	26	13
1.5	875	56	21
2.0	1250	87	28
2.5	1625	117	38

Table 2: Rough estimate of the feed derived waste production/crop/ha  
(Production 2 tonnes/ha: FCR 1.5)

	1 <sup>st</sup> M Kg.	2 <sup>nd</sup> M Kg.	3 <sup>rd</sup> M Kg.	4 <sup>th</sup> M Kg.
Feed offered	120	510	960	1410
Waste production				
Organic matter	70	298	560	822
Nitrogen	5	20	36	52
Phosphorus	2	7	14	20

### **Biotechnological interventions:**

Biotechnological interventions in aquafeed development include nutrient enrichment through solid state fermentation; production of B group vitamins, essential amino acids like lysine, novel methods of production of essential fatty acids like docosahexaenoic, eicosapentaenoic and arachidonic acids, and carotenoids. Microbial and enzyme mediated bioconversion of raw materials have been shown to improve digestibility and nutrient bioavailability and reduction in waste output. The most significant impact is in breakdown of antinutrients and fibre levels in plant products.

### **Conclusion**

There is increasing concern worldwide about the decline in world fishmeal production and the availability of adequate quantities of animal-based raw materials and key micro ingredients for meeting the demands of the growing aquafeed industry. In future biotechnological interventions will have greater role in aquafeed formulation and production for sustainable fish and crustacean aquaculture.