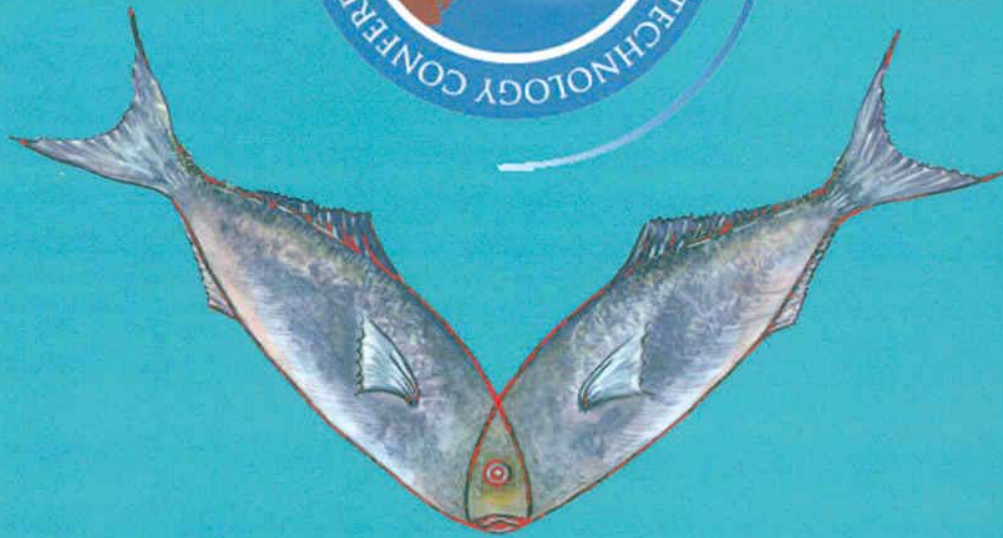


SEAFOOD QUALITY AND SAFETY

Advances in the New Millennium



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CHAPTER 28

AQUA FEED: RESEARCH CHALLENGES AND FUTURE TRENDS

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Management for sustainability in aquaculture involves several aspects, including aqua feed production and feeding strategies. This contribution deals with studies done in several laboratories, both in America and Europe, relating to the quality of the protein ingredients used to produce feed for aquafarming. The modeling of the digestive systems of fish and crustaceans and the use of *in vitro* techniques for understanding the biochemical physiology of organisms intended for aquafarming and evaluating the presence of inhibitors of digestive enzymes in feed ingredients is at the Heart of this issue. Also, the quality of currently-used raw materials, processes of preparation, products available, and the use of alternate ingredients are discussed.

INTRODUCTION

According to the FAO, a major challenge for aquaculture is to maintain, and where sustainable, enhance the contributions made to global fish supplies. In 1996, aquaculture contributed 26.5% of the world's fish production, becoming the fastest growing food production sector in agriculture. Moreover, aquaculture makes a major contribution to global food security, and opportunities still exist to expand its role.

Management for sustainability in aquaculture involves several aspects, including aqua feed production and feeding strategies. The expected increase in aquaculture productivity will require adequate feed, environment care, quality of the product, and profit. Production of feed for aquatic organisms is a current challenge, both scientifically and technically. Aqua feed is made from trash fish, fishmeal, and some slaughterhouse leftovers, as the main source of protein for feeding carnivores, omnivores, and even herbivores. Such protein feed practices

generate many criticisms that have to be solved to support sustainable aquafarming activities in the 21st Century.

Usually, a high concentration of protein is used in feed. Up to 7 kg of caught fish is needed to produce one kg of farmed organisms. There are severe discrepancies in the lowest feed protein concentrations that yield acceptable performance (usually called the optimum) of farmed organisms. All of this is related to the quality of protein ingredients used to fabricate feed. It is a common practice to produce fish or slaughterhouse leftover meals by a high temperature process during the drying of the product before packing. This process affects the nutritional quality of the product by reducing the bioavailability of amino acids and increasing ash content. Additionally, a possible embargo of feed produced from animal-derived ingredients is shadowing the encouraging panorama of aquafarm feeding technologies.

This chapter deals with studies done in several laboratories, both in America and Europe, relating to the quality of the protein ingredients used to produce feed for aquafarming. The modeling of the digestive systems of fish and crustaceans and the use of *in vitro* techniques for understanding the biochemical physiology of organisms intended for aquafarming and evaluating the presence of inhibitors of digestive enzymes in feed ingredients is at the heart of this issue. Also, the quality of currently-used raw materials, processes of preparation, products available, and the use of alternate ingredients are discussed.

The current status and challenges of aqua feeds

Feeds are used for a variety of organisms, both terrestrial and aquatic. The biggest demanders for feed are the poultry and pig industries with 32 and 31% of the total, respectively. Dairy and beef industries demand 17 and 11%, respectively. Aqua feeds constitute about 3% of the total. Aquaculture competes for feed ingredients with traditional farming. Aqua feed production in 1994 was 4,250,000 tons (Smith and Guerin 1995), with Asia consuming about 60% of the total and North America about 10%. Most of the feed produced is intended for salmonids and shrimps (27 and 25%, respectively). The feeding habits of aqua farmed organisms are diverse. Some, the photosynthetic ones, only need sunlight and minerals. But most of them, omnivorous and carnivorous, need a food supply, even herbivores are fed formulated feeds, including animal protein ingredients to increase the yield and reduce time of nurture.

Fish meal is the most widely used protein ingredient in feed formulations, with more than a million tons produced in 1994. Poultry is the largest demanding sector (Pike, 1997). Aqua feeds use 17% of total fish meal production. The need for fish meal in 2010 is estimated to be more than a

million and a half tons, about a 50% increase in 16 years. Aqua feeds will demand 23% of the total production in 2010, an increase of 6% in 16 years.

However, the total production of fish meal may not satisfy the demand, because the total production has remained unchanged between 1961 and 1995. Worsening the situation is the fact that most fish meal producers are burning the fish proteins because of obsolete methods of fishing, managing, and industrialization, especially during the drying process. The process, from a nutritional point of view yields a poor product. Moreover, environmental issues and rational use of marine harvests are of interest. Such issues are common in environmental and political arenas. It has been said that fish feed is exhausting the oceans. Thus, new findings on unintended impacts of fish farming that put both oceans and the aquaculture industry at risk.

Recent developments in aqua feed

Aqua feeds are under the spot light for several reasons. Increasing demand, the search for improved nutritional characteristics, abuse of resources (mainly from ignorance), operational problems (like inefficient processing and the presence of natural anti-nutritional compounds in raw material), and political pressures are challenging issues, both scientifically and technologically.

On the other hand, aquaculture stands for opportunities for development if scientifically sound techniques for production of food are used. Aqua feeds lie at the intersection of fisheries industrialization and aquafarming. Opportunities are open to both the developed and developing countries.

Several techniques have been developed to study the efficiency of formulated feed for particular organisms. Two variables are most important, digestibility of the protein and the food-biomass conversion ratio. There are *in vivo* techniques, which are tedious and expensive because they involve dozens of organisms, replicate and feeding, sampling, and analyzing residual feed and feces. Alternative *in vitro* techniques are also available. A technique has been developed to evaluate protein digestibility for salmonids (Dimes and Haard, 1994). An *in vitro* technique to evaluate protein digestibility for farmed shrimp has also been developed at the CIBNOR. The technique evaluates the degree of hydrolysis of protein by enzymes from the digestive system of the studied organism, and involves the evaluation of the progress of protein hydrolysis using a pHstat. A correlation between the quality of the protein source and digestibility, in which the poorest the quality of the ingredient, the lowest the DH was found (García-Carreño *et al.*, 1997; Ezquerro *et al.*, 1997). We also found a correlation of 0.77 between digestibility obtained in the *in vivo* assay and the degree of hydrolysis in the *in vitro* assays. This allows the use of *in vitro* techniques to evaluate the digestibility of ingredients, feeds, and the effect of

processing on the quality of the product (Ezquerria *et al.*, 1997). Some plant protein ingredients possess antinutritional compounds such as lectins, toxic amino acids, allergens, and proteinase inhibitors. Such inhibitors can reduce the proteolytic activity of enzymes from the digestive gland of shrimp. Inhibitors in several legume seeds reduced up to 60% the total proteolytic activity from the digestive proteinases of white and brown shrimp (*Penaeus vannamei* and *P. californiensis*). The effect is species specific, the enzymes from the brown shrimp being most affected (García Carreño *et al.*, 1997). Fortunately, there are procedures to reduce inhibitory activity of protease inhibitors. An increase in the degree of hydrolysis of protein in legume seeds is shown when treating the seed protein extracts are heated at 85°C. The response to the process is also species specific, both for the legume seeds and shrimp.

At CIBNOR, there is interest in demonstrating that better protein ingredients will yield improved performances in farmed organisms. We have evaluated the supplementation of a commercial shrimp feed with commercial fish protein hydrolyzate, krill protein hydrolyzate, and a squid concentrate produced in the lab (Córdova Murueta and García Carreño, 2001, 2002). The final weight and food conversion ratio of groups of shrimp fed with the supplemented feed at concentrations of 3, 9, and 15%. Final weight was higher in all treatments, except in squid fed at 15%, when compared to the control group that was fed with commercial feed. Food conversion rates were improved in all the treatments, except for fish hydrolyzate at 15% and squid at 9 and 15%. All treatments at the lowest supplementation level of 3% improved the performance of shrimp.

Variables of protein digestibility, both *in vivo* and *in vitro*, like apparent digestibility coefficient (ADC), regardless of the protein ingredient, were significantly improved in all the experimental groups fed with supplemented feeds (Córdova and García Carreño, 2002). Shrimp digested the supplemented feeds better. In the *in vitro* approach to analyzing the degree of hydrolysis by digestive enzymes indicated that shrimp fed with supplemented feeds exhibited a higher degree of hydrolysis than that of the control group. Also, the degree of hydrolysis of supplemented feeds showed better digestion when using commercial enzymes. Thus, feeds were substantially improved when supplemented with better protein ingredients

When feeding piracanjuba, a fish from Brazilian rivers, the final weight was higher in specimens fed with an experimental feed than the specimens fed on a commercial feed. The weight of fatty tissue was higher in the experimental group. This is important because, at the time of assay, these fish were preparing for migration upstream to reproduce, and fatty tissue is the reserve of energy for the migration (García Carreño *et al.*, 2002). For the piracanjuba, the digestibility of casein by enzymes from this fish fed on commercial and experimental feeds

was significantly different. The degree of hydrolysis, when using the four enzyme cocktail of Hsu *et al.* (1977) was lowest. The degree of hydrolysis, when using the enzymes from piracanjuba fed the commercial feed, was lower than when using enzymes from piracanjuba fed with experimental feed. The quality of feed, beyond nutritional factors, definitely affects variables in the metabolism of farmed organisms. Modification of digestibility of food and feed by modulator-supplemented feeds is expected in future research.

Because the availability of animal protein ingredients is not enough to satisfy demand and health-associated problems, the use of alternative protein ingredients must be assessed. One alternative is plant protein ingredients, but the presence of anti-physiological compounds, such as digestive enzyme inhibitors, are among drawbacks that need to be considered. Even some animal protein ingredients possess enzyme inhibitors. A cheap, fast, and easy methodology was developed to evaluate the effect of inhibitors present in protein ingredients on the digestive proteases of aquafarmed organisms (Alarcón *et al.*, 2001). Extracts of digestive enzymes from the organism and plant ingredients under study were tested for their inhibitory effects. The enzymes and the test ingredient were mixed and the mixtures assayed for proteinase activity, using casein as a substrate. The absorbance was transformed to a percentage when the control of no inhibition was set at zero inhibition. Also, the protein, enzyme, and inhibitor composition were evaluated using a substrate-SDS-PAGE. Both animal and plant protein ingredients yielded some degree of inhibition for digestive proteinases of farmed organisms. The Mediterranean seabream, *Sparus aurata* was inhibited up to 19% of the total proteinase activity by a commercial fish meal. Ovalbumin inhibits more than half of the total activity. Plant ingredients are good inhibitors for *S. aurata* enzymes, which means that anti-physiological compounds in feed can eliminate a good deal of the total enzyme secreted to digest food protein. Commercial microencapsulated feed for *S. aurata* fingerlings were fabricated using ovalbumin as binder. The feed inhibited 22% of the total proteinase activity of the fish, one of the reasons why the use of microcapsules to fully substitute live food has failed. The problem is that food technologists have to deal with ingredients that have naturally occurring inhibitors.

Enzyme extract from four shrimp species were inhibited to varying extents by both animal and plant protein ingredient extracts. To understand how digestive enzymes were affected, the effect of inhibitors on enzyme extracts of three fish was calculated. The response to inhibition factors was species specific, and followed particular kinetics and extents. By using a technique that combined the evaluation of the degree of hydrolysis, electrophoresis, and lane densitometry to follow the reduction in size of the main proteins in ingredients and feed, it is possible to calculate a coefficient of protein degradation (CPD).

The algorithm allowed calculation of the maximum concentration of a particular proteinase inhibitor containing ingredient in a feed with a negligible effect on digestive enzymes.

$$CPD = \sum_{i=1}^n \left[\frac{A_i(t=0) - A_i(t=90\text{min})}{A_i(t=0)} \times 100 \right] \frac{A_i(t=0)}{\sum_{i=1}^n A_i(t=0)}$$

Trends

Efforts in the near future should be directed at:

- 1) Reducing the amount of fishery resources used by producing improved protein ingredients.
- 2) Improving the performance of farmed organisms by using better feed, including growth factors and nutraceuticals.
- 3) Using aqua feed to support sustainability in aqua farming.

All these goals are possible, if a strong science of aqua feed production is developed.

Farmers have to be educated to understand that the health of the farm depends on a deep knowledge of organisms and inputs to the farm. One should also recognize that most aquaculture problems result from trying to push a balanced ecosystem too far, in the interests of higher production or higher profits.

REFERENCES

- Alarcón, J.; García-Carreño, F. and Navarrete del Toro, M. 2001. Effect of plant protease inhibitors on digestive proteases in two fish species with aquafarming potential. Pacific Fisheries Technologists. 53 Annual Meeting, La Paz, BCS. México, PFT.
- Córdova Murueta, J. and García Carreño, FL. 2001. The effect on growth and protein digestibility of shrimp *Penaeus stylirostris* fed with feeds supplemented with squid (*Dosidicus gigas*) meal dried by two different processes. *J. Aquatic Food Prod. Technol.* 10: 35-47.
- Córdova-Murueta, J. and García-Carreño, FL. 2002. Nutritive value of squid and hydrolyzed protein supplement in shrimp feed. *Aquaculture* 210: 371-384.
- Dimes, L. and Haard, F. 1994. Estimation of protein digestibility-I development of an in vitro method for estimating protein digestibility in salmonids (*Salmo gairdneri*). *Comp. Biochem. Physiol.* 108A(2/3): 349-362.

- Ezquerro, M. and García-Carreño, FL. 1997. pH-stat method to predict protein digestibility in white shrimp (*Penaeus vannamei*). *Aquaculture* 157: 251-262.
- García-Carreño, FL.; Navarrete, M. and Ezquerro, M. 1997. Digestive shrimp proteases for evaluation of protein digestibility in vitro. I: Effect of protease inhibitors in protein ingredients. *J. Marine Biotechnol.* 5: 36-40.
- García-Carreño, FL.; Albuquerque-Cavalcanti, C.; Navarrete del Toro, MA. and Zaniboni-Filho, E. 2002. Digestive proteinases of *Brycon orbignyanus* (Characidae, Teleostei): Characteristics and effects of protein quality. *Comp. Biochem. Physiol.* In press.
- Hsu, H.; Savak, D.; Satterlee, L. and Miller, G. 1977. A multienzymatic technique for estimating protein digestibility. *J. Food Sci.* 42: 1269-1273.
- Pike, I. 1997. Future supplies of fish meal and fish oil: quality requirements for aquaculture with particular reference to shrimp. In *Feed Ingredients Asia '97*. Uxbridge, Turret-RAI, pp. 26.
- Smith, P. and Guerin, M. 1995. Aquatic feed industry and its role in sustainable aquaculture. In *Feed Ingredients Asia '95 Conference*. Uxbridge, Turret-RAI, pp. 1-11.