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**Optimization of Diet for Post Larval/Juvenile Sea
Bass and Hybrid Striped Bass Based on Enzymatic
Profiles of their Digestive Tracts**

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**Title: OPTIMIZATION OF DIET FOR POST
LARVAL/JUVENILE SEA BASS AND HYBRID STRIPED BASS
BASED ON ENZYMATIC PROFILES OF THEIR DIGESTIVE
TRACTS.**

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Abstract

Research Title: Optimization of diet for postlarval/juvenile sea bass and hybrid striped bass based on enzymatic profiles of their digestive tracts.

The overall goal of this research work was to identify the main proteolytic activities which take place in the digestive tracts of young bass fish, and use the knowledge acquired in order to improve feed protein utilization in juvenile fish based on their digestive capacity.

The results of the work clearly showed that the young fish possess the entire profile of proteolytic enzymes which is found in adult fish. Yet, in the young fish the level of activity is substantially lower per gram tissue (or gram protein) as compared with the activity found in the digestive tracts of the same fish at an older (larger) age. In addition it was found that the main proteolytic enzyme in these fish is chymotrypsin which accounts for almost 80% of the proteolytic activity. An effort aimed at enhancing this activity has lead to the interesting finding that alcohol substantially enhances the proteolytic activity of fish intestines.

Fish intestinal homogenates were used in order to evaluate the suitability of various feeds for the fish. Potential feed proteins were subjected to the proteolytic activity of the fish enzymes in vitro, in a manner simulating the natural process. The proteolytic activity was monitored by the evaluation of the products, i.e. amino acid released. This method has proved to be a powerful tool which enables us to predict with a very high degree of accuracy the potential of a feed to promote growth. Selection of feed based on the proteolytic capacity of the fish digestive tracts can now be implemented in feed formulation, as anticipated in the original research proposal.

Overview including: Objectives of the original research proposal and the evaluation of research achievement with respect to the original research proposal.

The overall goal of this research work was to identify the main proteolytic activities which take place in the digestive tracts of young bass fish and use the knowledge acquired in order to improve feed protein utilization in juvenile fish. Thus, alleviating the mass mortalities related to weaning the young fish from live feeds while finding suitable artificial feeds for them. Obviously the feed proteins are not the only factor affecting the growth and survival of young fish. Yet, since the proteins constitute above 50% of the diet and are the major building blocks of the body their importance is crucial.

Most of the published research work carried out on juvenile fish nutrition did not concentrate on taking the "fish eye's view" i.e. surveying the proteolytic capability of the fish and finding the most suitable food to accommodate it. It was assumed that when the live food organisms are consumed they "donate" their proteolytic enzymes to the fish and thus compensate for the lack of some enzymes in the fish.

During the first stage of the research, efforts were devoted mainly to studying the various activities related to proteolysis in the gastrointestinal tracts of both sea bass and hybrid striped bass, in accordance with the research plan suggested in the original proposal. The general knowledge of the various gastrointestinal activities and their detailed properties are a prerequisite for optimizing the diet according to the digestive capacity of the fish.

The first stage research activity was therefore devoted to adapting the various measuring methodologies of the enzymatic activities of interest to the homogenates of various parts of the fish gastrointestinal tracts. These were tested during the fish developmental stages as well.

Phase one of the research plan, i.e. characterization of the proteolytic activity profiles in the digestive tracts of sea and hybrid striped bass was successfully accomplished. The results of the work clearly showed that the young fish possess the entire profile of proteolytic enzymes which is found in adult fish and do not lack any of the enzymes. On the other hand the results showed that in the young fish the level of activity is substantially lower per gram tissue (or gram protein) as compared with the activity found in the digestive tracts of the same fish at an older (larger) stage. In addition it was found that the main proteolytic enzyme in these fish is chymotrypsin which accounts for almost 80% of the proteolytic activity.

Based on these results we devised an assay which included the use of fish intestinal homogenates in order to evaluate the suitability of various proteins as feed ingredients for the fish. The potential proteins were subjected to the proteolytic activity of the fish enzymes in a manner simulating the natural process and the proteolytic activity was monitored by the evaluation of the products i.e. amino acid released. This method of evaluation has proved to be a powerful tool which enabled us to predict with a very high degree of accuracy the potential of a feed to promote growth, based on an in-vitro exposure followed by the examination of the proteolytic products. Actual growth trials under large tank conditions, which were run in order to corroborate the in-vitro results showed a remarkably high correlation

between the predicted and actual growth of the young fish. Screening potential feeds/proteins using the above described method is faster and more accurate than conducting laborious growth trials. Adaptation of feed to the proteolytic capacity of the fish digestive tracts can now be used in feed formulation, as anticipated in the original research proposal.

Following the successful completion of the first stage we began to search for various methods to enhance proteolytic activity within the young fish digestive tracts. It was found that by adding alcohol to an intestinal homogenate it is possible to substantially enhance the proteolytic activity. Furthermore, different alcohols produced varying levels of activity enhancement which was found to be strongly correlated with the length of the alcohol's chain. Of the various alcohols tested n-propanol showed the most promising results as it is less toxic and the level needed for maximal activity enhancement is relatively low.

Another way to enhance feeding and growth was the use of chemical attractants. It was found that exposure of the young fish to a number of attractants in the form of amino acids enhanced their growth. It is not clear whether this exposure enhances proteolytic activity or is responsible mainly for better food engulfment.

Relevant data, methodology, results and discussion

The results of the different experiments conducted in the course of the entire research project are summarized as manuscripts. Three of the manuscripts presented have already been published and the others are at various stages of the publishing process. Each of the manuscripts includes the methodology used and discusses the results and their implication.

Description of the Cooperation

The research teams in both countries were in constant communication throughout the entire research period. The constant flow of information provided both teams with better insight during the planning and execution of the various experimental procedures. The meetings held half way through the project enabled the careful evaluation of the results achieved up to that period and meticulous planning of the remainder of the experimental work.

Amino acid analysis of the tests run in both countries was carried out in the United States, as the facilities there were better equipped for this purpose.

The working relationship established in the course of this project will undoubtedly lead to future scientific cooperation between the research teams.

Conclusions

The overall results show that by exploring the proteolytic capabilities of bass fish it is possible to find proteins which are digested better by these fish thus achieving enhancement of growth and survival through the use of more suitable feeds.

Early stages of fish were not found to lack important proteolytic enzymes and their profile is identical to that of adult fish. The proteolytic activity level in the young fish was substantially lower on a per gram basis when compared with the adults. Improving the proteolytic capabilities of the young fish can be done by adding exogenous enzymes to the feed (taking into account the fact that they have to reach the intestines). In this case our recommendation will be to use chymotrypsin as this was found to be the enzyme responsible for an overwhelming proportion of the proteolytic activity. The alternative way would be to enhance the activity of the fish intestinal proteolytic activity. One way of doing so has been demonstrated in the course of this study. The interesting finding that alcohol enhances the proteolytic activity of fish intestines still remains to be tested in growth trials. The major problem is the delivery of the alcohol to the intestine without it being absorbed in the stomach. This can be achieved by using microencapsulated vesicles of alcohol which will only release their content when reaching the intestines. Chemoattractants can also be used in order to enhance feeding and growth in young fish as has been shown in this study.

The use of a biochemical test which will evaluate the suitability of proteins or new feeds for bass fish is a powerful tool which can save time and effort providing an accurate screening procedure.

The major findings of this study can be directly applied by farmers engaged in the production of early stages of bass fish. It is our hope that the findings will indeed be utilized in order to achieve better production of young fish since the world demand for juvenile fish required for aquaculture is growing at a rapid pace.

List of publications resulting from the research project

Publications in reviewed journals

Eshel, A., P. Lindner, P. Smirnoff, S. Newton and S. Harpaz (1993). Comparative study of proteolytic enzymes in the digestive tracts of the European sea bass and hybrid striped bass reared in freshwater.

Comp. Biochem. Physiol. 106A: 627-634.

Harpaz, S., A. Eshel and P. Lindner (1994). Effect of n-propanol on the activity of intestinal proteolytic enzymes of the European sea bass *Dicentrarchus labrax*.

J. Agricult. Food Chem. 42: 49-52.

Lindner, P., A. Eshel, S. Kolkovski, A. Tandler and S. Harpaz (1995).

Proteolysis by juvenile sea bass (*Dicentrarchus labrax*) gastrointestinal enzymes as a method for the evaluation of feed proteins.

Fish Physiology and Biochemistry 14: 399-407.

Manuscripts pending publication

Hughes, S.G. Tests of a microencapsulated amino acid diet for larval and juvenile striped bass.

Hughes, S.G. Effect of supplemental dietary crystalline amino acids on the feed consumption of juvenile striped bass.

Hughes, S.G., G.W. Vandenberg and J. Theriot-Hubert. Development of proteolytic enzyme activity throughout the digestive tract of hybrid striped bass (*Morone chrysops* x *M. saxatilis*).