嘉南藥理科技大學專題研究計畫成果報告

餵食蛋白酵素對石斑幼苗之生長影響

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Effects of survival on *Epinephelus* sp. larvae fed on protease supplemented diet

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中文摘要

此實驗在於以一種蛋白酵素添加至基本餌料，然後餵食幼石斑（14天大）以評估其生長與存活。在28天之餵食後，基本餌料組與蛋白酵素添加餌料組之生長與存活率並無顯著之差異，而活豐年蝦餌料餵食幼石斑在存活率（66.6％）或體重（60.2 mg）與皆有最佳之表現。餌料中之蛋白酵素失去活性可能導致幼石斑在28天基本餌料與蛋白酵素添加餌料之餵食試驗間無顯著生長與存活差異。

關鍵詞: 石斑、蛋白酵素、存活率、幼魚苗。

ABSTRACT

A bacterial protease was supplemented to a basal diet and fed to the 14 day-old grouper (*Epinephelus* sp.) larvae for the evaluation of growth and survival. After a 28-day feeding, survivals or body weight of the grouper larvae were no significant difference between the basal diet and protease supplemented diet. Live *Artemia* nauplii diet fed to the larvae gave the best performance either on final percentage survival (66.6%) or body weight (60.2 mg). The inactivity of protease enzyme in the diet may cause the no significant difference for the growth and survival between protease supplemented diet and basal diet in the 28-day feeding trial.

Keywords: Grouper larvae, *Epinephelus* sp., protease, survival.

INTRODUCTION

The grouper (*Epinephelus* sp.) is an important recreational and commercial fish species in Taiwan. Due to its high economic value, aquatic farmers have been made to culture this valuable fish species through private and governmental hatcheries in order to enhance populations.

Live zooplankton, especially live brine shrimp (*Artemia*) nauplii, usually provide the best performing diet for rearing the larval stages of fish and crustaceans, since it is easily obtained or cultured, has an appropriate size and is nutritionally adequate (Simpson et al. 1983; Leger et al. 1986). However, various reports have stated that using *Artemia* nauplii resulted in

poor larval rearing, e.g., chlorinated hydrocarbon contamination (Olney, 1980) or lack of essential fatty acids (Schauer et al. 1980; Leger et al. 1985). Artificially formulated diets were thus subsequently essential and important for incorporation of nutrient requirements for aquatic animals.

Usually, the intestinal tract of fish larvae is more simple and shorter than that of the adults and the larval digestive systems are not fully developed. According to Kainz (1976), insufficient digestive enzymes may be the reason that some fish larvae have difficulty digesting food. Govoni et al. (1986) also indicated that the activity of digestive enzymes is low at the first feeding and increases during the larval period before transformation. Hence, the addition of exogenous enzymes may help to increase the digestibility of macronutrients, especially for the larval fish in which the enzymatic systems are not fully established.

Dabrowski and Glogowski (1977) reported that proteolytic enzyme activity increased considerably when common carp fry were provided with bovine trypsin in their diets. Other studies also indicated exogenous enzymes contributed by fish food diets could advantageously influence fish larval growth and food utilization (Hjelmeland et al.1988; Pedersen et al. 1987; Sargent et al. 1979). However, attempts at adding exogenous digestive enzymes to formulated diets had shown little or no effect on the acceleration of the digestive processes of the fish larvae.

However, effective utilization of nonliving diets for the growth of this larvae has not yet been successfully achieved (. Thus, this study was carried out to evaluate the growth and survival rates of grouper larvae fed with a protease supplemented diet and a commercial diet in order to provide a better and more reliable diet to rear grouper larvae.

MATERIALS AND METHODS

Filtered seawater (0.45 μm) of 30 ‰ salinity was obtained from a local aquaculture farm. One gram of each dry *Artemia* cysts, RAC *Artemia* (Reference *Artemia* cysts) was separately hatched in a separatory glass funnel containing 2 L of filtered seawater (30‰ salinity) under continuous strong aeration at 25 ± 2°C for 24-h with light. After 24-h hatching, *Artemia* nauplii were harvested under a 106 μm sieve and transferred to 10L glass tanks containing seawater. Fifty grouper at two-wk-old (obtained from a local aquaculture farm) were randomly collected and transferred to each of the 10L glass tanks under moderate aeration (20 ± 2°C). All the treatments were conducted replicated twice.

Table one shows the composition of the ingredients used in this experiment for both the basal diet and protease supplemented diet. The individual dry ingredients were weighed, mixed and ground. Menhaden oil was then pipetted into the dry mixture and blended for several minutes. A volume of water half that of the dry mixture was slowly added to the slurry. This semi-moist slurry was then

extruded through a heavy-duty meat grinder. For the processing of the protease supplemented diet, a neutral bacterial protease (*Clostridium* sp., Alltech) at 0.05% of the total dry weight was spread on the top of the pellets, and then the pellets were passed through the grinder again. The two different pellet diets were then dried at 40°C with hot blowing air for 4 hr to obtain a constant moisture content. The dry pellets were then crumbled and mixed with a coffee grinder and passed through a 500 μm sieve to obtain 250-500 μm particles. These prepared diets were then stored in the -4°C freezer, ready for feeding the fish larvae.

A ration in dry weight of diets of 100% of fish wet weight was used for each diet. After 28 days of feeding, fish larvae were collected, blotted dry and measured for their weight and length. Survival and growth conditions of the fish larvae were analyzed by one way analysis of variance and Duncan's multiple range test for the significant differences among the different diets.

RESULTS AND DISCUSSION

In Table 2, the final percent survival (42.6%) for protease supplemented diet is shown based on the numbers of the fish collected on the last day of the feeding divided by the original numbers of the fish used. This Table also shows the percent cannibalism of fish fed the protease supplemented treatment (7.2%) is not significantly different from *Artemia* (5.9%) and basal diet (6.1%) treatments. Percent cannibalism is expressed as the difference between the total mortality recorded and the initial stocked number of the fish in this experiment.

In most aquaculture hatcheries, an average survival of 30-40% is normal for striped bass larvae, compared to 10-20% survival during the early years of culture (Kerby, 1986). In this present study, larvae fed with *Artemia* nauplii showed superior growth & survival. Some unknown growth factors existing in nauplii may contribute to their superior performance in rearing striped bass larvae (Baragi & Lovell, 1986). A similar recent study was conducted in this laboratory based on the same ingredients supplemented with structurally different kinds of ascorbic acid. The percent survivals for the ascorbic acid trials ranged from 22.9-50.0% (Ashraf et al., 1992). The percent survivals for experimental diets in this present study (66.6-41.7%) were higher than that range.

The percent survivals in this study were all higher than the study by Webster & Lovel (1990). During that 19-day posthatch feeding experiment, survival for striped bass was greatest for fish fed with live *Artemia* (28.5%), followed by fish fed a dry diet (18.0%), freeze-dried *Artemia* (9.8%) and shock-frozen *Artemia* (9.3%).

Protease enzymes are present in the *Artemia* and can be functionally released in herring larvae after consumption. According to Grabner et al. (1981), when frozen or freeze-dried zooplankton material is introduced into water, the activity of protease and of L-lactate dehydrogenase is rapidly lost. The background protease

activity either in basal diet or protease supplemented diet may originally come from endogenous sources such as dietary bovine liver extract or yeast autolysate ingredients. The unstable enzyme supplement may also contribute to the lack of difference in growth, e.g. specific growth rate, final body weight or biomass index between basal diet and protease supplemented diet. According to various reports, cannibalism was frequently found in intensive culture or closed systems of striped bass larvae (Braid & Shell, 1981; Braid, 1974; Paller & Lewis, 1987). Inadequate food supplies may initially increase cannibalism in young larvae (Lewis & Heiginger 1975).

It is concluded that the basal diet used in this feeding trial can provide minimum satisfactory for the survival and growth of grouper larvae. An unprotected or non-immobilized bacterial protease supplement in the basal diet did not significantly improve the survival and growth performance for this fish during a 28-day feeding period. Based on a study by Maugle et al. (1983), digestive enzymes supplemented in a microencapsulated and thus immobilized form seem to have prevented inactivation during storage and resulted in increased growth for shrimp (*Penaeus japonicus*). The continuation of this research will explore the protection of the enzyme particles such as microencapsulated enzymes to retain activity during processing, storage and water.

Table 1. Percent of ingredients and proximate composition of the formulated diets in this feeding experiment.

Ingredients	%(dry basis)
Krill meal (58% Protein)	56
Whole egg solids	25
Plankton fines	5
Bovine liver extract	2
Yeast autolysate	2
Lecithin	2
Menhaden oil	3
Betaine	2
Sea salt	1.5
Gum binder	1
Trace mineral mixture	0.5
Vitamin mixture	1
Ascorbyl-2-polyphosphate	0.01
Sucrose	1
Protease (*Clostridium* sp., Alltech)	0.05

Table 2. Average final percentage survival and cannibalism of grouper larvae in this 28-day experiment.

Treatments	Final % survival	Final body weight (mg)‡	% Cannibalism†
Artemia	66.6[a]*	60.2[c]	5.9
Basal diet	41.7[b]	31.7[d]	6.1
Protease	42.6[b]	30.4[d]	7.2

*Different letters denote pairs of groups significantly different at the 0.05 level for Duncan's multiple range test.

†% Cannibalism = (initial # of fish-total # of mortality fish recorded)/initial # of fish.