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Effects of Dietary Enzymes on the Larval Performance of the Red Sea Bream (*Pagrus major*)

Julio López-Alvarado

Prometeo Researcher, Instituto Nacional de Pesca, Letamendi 102 y La Ría, Guayaquil, Ecuador

Abstract: ***In order to study the effect of exogenous dietary enzymes on the larval performance of red sea bream, 6 diets with 3 levels of Pancreatin (0, 0.05 and 0.1 % of the diet)and 2 levels of dietary crystalline amino acids (4 and 14 % of the diet) were fed to red sea bream larvae. Larvae fed diets with 0.1 % Pancreatin had the highest specific growth rate. The survival was better for larvae fed diets with intact protein than for larvae fed diets with crystalline amino acids. The supplementation of Pancreatin to diets with intact proteins increased larval performance. However, diets with crystalline amino acids did not benefit from Pancreatin supplementation. For larval diets with high levels of intact proteins, supplementation of the diets with 0.05 % Pancreatin is recommended, while its use is discouraged for larval diets with high levels of free amino acids.***

Keywords: dietary enzymes, larval diets, red sea bream

1. Introduction

In the rearing of marine fish larvae, a lot of effort has been placed in developing an artificial microdiet to substitute the need for live food. Many studies have been carried out using different types of microdiets [1]–[5]. It has been suggested that one of the reasons for the low performance of these diets, compared to live foods, is the limited ability of the larvae to digest these microdiets. Fish larvae have poorly developed digestive systems, and an exogenous source of enzymes may be needed to improve the digestion of the microdiets. The protein wall of microencapsulated diets was broken down in the larval intestine when rotifers were present in the diet [6], suggesting the need of an exogenous source of enzymes to break down the protein molecules.

The role of exogenous enzymes in the digestion of fish larvae has been discussed by several authors [7]–[8]. The application of proteolytic enzymes to larval feeds was carried out as early as 1977 [9]; and in 1991 porcine enzymes were applied to sea bream feeds [10]. In these experiments, the addition of enzymes to the diets did not have an adverse effect on the survival of the larvae, and the utilization of the dietary protein was enhanced.

The aim of this research was to assess the effect of dietary enzymes on the larval performance of red sea bream.

2. Materials and Methods

2.1 Diets

Zein microbound diets were prepared following a method modified from Teshima et al. [11]. The composition of the diets is shown in Table 1. Six experimental diets were tested, all with approximately the same amount of each essential amino acid, and a protein level of 60 %. Three levels of Pancreatin (Sigma) were used: 0.00, 0.05 and 0.10 % of the diet. Two sets of diets were prepared one with 4 % and one with 14 % crystalline amino acids in the diet.

Table 1: Composition of microdiets used (g/100 g dry wt.)

Ingredient	*Diet 1*	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*	*Diet 6*
casein	30.00	30.00	30.00	20.00	20.00	20.00
fish meal	20.00	20.00	20.00	20.00	20.00	20.00
Krill meal	10.00	10.00	10.00	10.00	10.00	10.00
EAA	3.00	3.00	3.00	7.00	7.00	7.00
NEAA	0.00	0.00	0.00	6.00	6.00	6.00
vitamins	6.00	6.00	6.00	6.00	6.00	6.00
minerals	5.00	5.00	5.00	5.00	5.00	5.00
fish oil	10.00	10.00	10.00	10.00	10.00	10.00
dextrin	6.70	6.65	6.60	6.70	6.65	6.60
attractants	1.30	1.30	1.30	1.30	1.30	1.30
zein	8.00	8.00	8.00	8.00	8.00	8.00
Pancreatin	0.00	0.05	0.10	0.00	0.05	0.10
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00

2.2 Fish and Culture Conditions

Eggs from red sea bream (*Pagrus major*) were received from Kagoshima Prefectural Fisheries Research Center. The eggs were placed in a 500-l tank wit seawater at a temperature of 17ºC, with gentle aeration and circulation of water. Two days after hatching, rotifers were fed to the larvae. Ten days after hatching the larvae were transferred to 12 100-l tanks, at a density of 1000 larvae per tank. The microdiets were fed every half an hour during the morning, and every hour during the afternoon. The appropriate amount of feed was hydrated with sea water in a beaker, and delivered to the tanks. In the evening, rotifers were fed to the tank at a density of 5 rotifers/ml, to provide food during the night. The particle size of the diets was increased during the feeding period, from a size of less than 125 µm to a size of 250 µm. Growth was estimated every 10 days, measuring a sample of 10 larvae from each tank. Survival was estimated every second day, counting the dead larvae siphoned from the bottom of the tanks.

2.3 Analytical Methods

At the end of the experimental trials, samples from all treatments were taken, and tissue and dietary amino acids were determined using a Shimadzu HPLC after hydrolysis of

the protein at 110 ºC for 22 hours with methane sulphonic acid. N-leucine was used as internal standard. The crude protein of the diets were determined using the Kjeldhal method. All data were analysed using Statview.

3. Results

3.1 Growth

Growth of the larvae fed the six experimental diets is presented in figure 1. Groups fed diets 3 and 6, with 0.1 % Pancreatin, had the highest final length and growth rate, significantly higher than for the rest of the groups. Diets 2 and 5, with 0.05 % Pancreatin, produced a lower growth rate than the control group. The lowest growth rate was produced by diet 4, with 14 % crystalline amino acids and no Pancreatin.

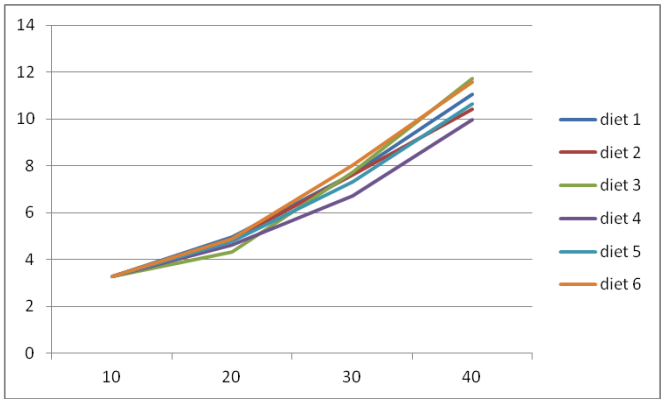

Figure 1: Mean body length (mm) of the larvae fed the different diets after 10, 20, 30 and 40 days

3.2 Survival

Survival of the larvae fed the different diets is represented in figure 2. The highest survivals were for the larvae fed diets 1, 2 and 3, with 4 % dietary crystalline amino acids. The larvae fed diets with 14 % crystalline amino acids had a lower survival.

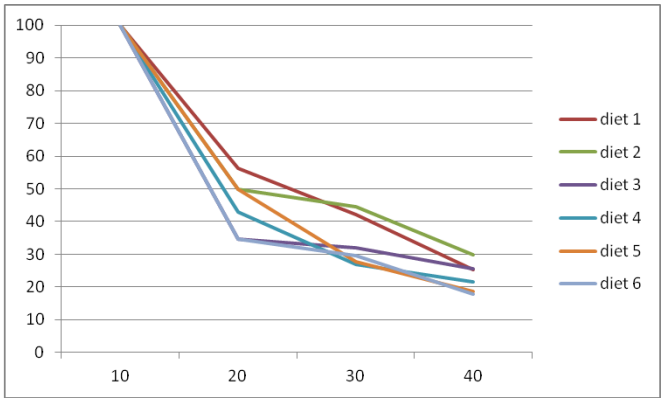

Figure 2: Survival of red sea bream larvae fed the different diets after 10, 20, 30 and 40 days

3.3 Larval Performance Index

To make a better assessment of the larval performance, Larval Performance Index (LPI) was used:

LPI= SGR * survival

The results of SGR´s and LPI´s are presented in Table 2:

Table 2: Specific Growth Rate and larval performance index of larval red sea bream fed diets 1-6.

Diet fed	*Specific Growth Rate**	*Larval Performance Index***
1	4.01 ± 0.39^{b}	101.95 ± 9.95^{c}
2	3.82 ± 0.40^{c}	114.24 ± 11.99^{a}
3	4.20 ± 0.49^{a}	107.64 ± 12.44^{b}
4	3.66 ± 0.49^{c}	79.04 ± 10.49^{d}
5	3.89 ± 0.41^{c}	72.31 ± 7.67^{e}
6	4.17 ± 0.44^{ab}	73.72 ± 7.72^{e}

*SGR= $\frac{\ln(\text{final body length}) - \ln(\text{initial body length}) * 100}{\text{Time in days}}$

** LPI=SGR * survival

In terms of LPI, diet 2 was the best. LPI is significantly higher in larvae fed diet 2 than in the rest of the experimental groups. Diets 3 and 1 had a good LPI, higher than 100, that indicates that the diets fed are acceptable. Diets 4, 5 and 6 had LPI much lower than 100, and all are significantly lower than for the control group (diet 1).

3.4 Amino Acid Analysis

The total amino acids in the diets and larvae at the end of the experiment are presented on tables 3 and 4. The amino acid profiles in all diets are very similar, as intended in the experimental design. In the fish body, the amino acid profiles are also very similar among all the groups.

Table 3: Total amino acids in the diets (g/100 g dw)

Amino acid	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Tau	0.22	0.23	0.24	0.27	0.23	0.22
Hypro	0.06	0.05	0.02	0.04	0.04	0.05
Asp	2.92	2.96	2.74	3.35	2.91	2.93
Thr	1.50	1.56	1.41	1.36	1.21	1.17
Ser	1.97	1.88	1.78	2.21	1.82	1.76
Glu	8.92	9.16	8.55	10.14	8.61	8.15
Pro	3.60	3.60	3.42	4.16	3.86	3.53
Gly	1.31	1.37	1.33	1.43	1.23	1.26
Ala	2.11	2.21	2.07	2.36	2.07	2.02
Cys	0.04	0.06	0.04	0.06	0.07	0.08
Val	1.75	2.00	1.86	2.09	1.89	1.87
Met	0.82	0.39	0.34	0.56	0.52	0.64
Ile	1.64	1.87	1.75	1.98	1.79	1.72
Leu	4.96	5.06	4.80	5.64	4.81	4.63
Tyr	1.89	1.93	1.82	1.85	1.78	1.77
Phe	1.97	1.98	1.88	2.20	1.94	1.86
His	0.87	0.88	0.83	1.02	0.79	0.77
Lys	3.40	3.66	3.45	3.36	3.57	3.24
Trp	0.40	0.01	0.01	0.12	0.07	0.45
Arg	2.54	2.62	2.45	2.72	2.57	2.54

Table 4: Total amino acids in the larvae after 30 days feeding on the different diets (g/100 g dw)

Amino acid	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Tau	0.02	0.03	0.01	0.04	0.03	0.02
Hypro	0.19	0.15	0.27	0.22	0.17	0.15
Asp	4.11	4.25	4.01	3.77	4.33	4.25
Thr	1.84	1.97	1.85	1.77	1.97	2.02
Ser	1.80	1.90	1.84	1.74	1.86	2.11

Glu	7.44	7.83	7.24	6.77	7.84	7.72
Pro	2.31	2.27	2.31	2.13	2.18	2.15
Gly	2.50	2.68	2.67	2.36	2.54	2.63
Ala	2.50	2.71	2.56	2.29	2.82	2.85
Cys	0.25	0.01	0.01	0.21	0.14	0.01
Val	2.10	2.26	2.06	2.03	2.33	2.13
Met	0.99	0.81	1.00	1.33	1.01	1.36
Ile	1.88	1.99	1.80	1.82	2.02	1.84
Leu	4.09	4.37	4.00	3.85	4.48	4.33
Tyr	1.85	1.68	1.59	1.55	1.67	1.77
Phe	1.97	1.94	1.88	1.78	1.96	1.95
His	1.19	1.32	1.33	1.18	1.26	1.29
Lys	3.58	3.31	3.06	3.09	3.56	3.33
Trp	0.01	0.01	0.30	0.48	0.38	0.26
Arg	3.14	3.19	3.12	2.97	3.22	3.10

4. Discussion

The specific growth rate of the larvae fed diets 3 and 6, with 0.1 % Pancreatin, were significantly higher than for the other experimental groups (Table 2). However, survival of larvae fed diet 6 was quite low. In diet 6, only 50 % of the diet is composed of intact protein (casein, fish meal, krill meal) and the rest of the amino acids are given in crystalline form. It seems that with this level of intact protein in the diet, 0.1 % Pancreatin in the diet is too high, and too many enzymes are liberated in the larvae´s digestive tract. Pancreatin is a purified extract from porcine pancreas that contains many enzymes, including amylase, trypsin, lipase, ribonuclease, and protease. It can digest at least 25 times its weight of casein in 60 min at pH 7.5. At an inclusion level of 0.1 % of the diet, these enzymes may be partially digesting or causing lesions to the digestive tract of the larvae. However, in diet 3, also with 0.1 % Pancreatin in the diet, but with 60 % of the diet as intact protein, survival was high, similar to the survival of the control group fed a standard diet. With these levels of intact protein in the diet, 0.1 % Pancreatin in the diet is not detrimental to larval performance. The highest survival was achieved by larvae fed diet 2, with 4 % crystalline amino acids and 0.05 % Pancreatin in the diet. It is clear from these data that in order to justify the use of Pancreatin in larval diets, a large proportion of the diet must be in the form of intact protein. In diets including high levels of crystalline amino acids, the use of Pancreatin to increase the digestibility of the diets is not recommended, since part of the protein source is already in the free form, and adverse effects may appear.

The larval performance index was best for fish fed diet 2, with 60 % of the diet in the form of intact protein, and 0.05 & Pancreatin in the diet. Increasing the level of Pancreatin to 0.1 % did not result in any improvement in LPI. The LPI of diets 4-6, with 14 % crystalline amino acids in the diet, are all lower than the LPI of the control group (diet 1). The inclusion of Pancreatin in these diets (0.05 % in diet 5 and 0.1 % in diet 6) decreased the LPI due to reduced survival.

5. Conclusion

The use of Pancreatin as a source of digestive enzymes in fish larvae is recommended only when a high proportion of the protein is present in the diet in its bound form (intact protein). In that case, a level of 0.05 % Pancreatin in the diet is recommended, since higher levels may cause damage to the larvae´s digestive system. In diets including crystalline amino acids, the use of a dietary source of enzymes may cause mortalities due to an excessive enzymatic activity in the digestive tract.

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Author Profile

Julio López-Alvarado received a B.Sc. in Marine Sciences from Universidad de Las Palmas de Gran Canaria, Spain 1987, M.Sc. in Aquaculture from the University of Stirling, Scotland 1990 and Ph.D. in Fisheries from Kagoshima University, Japan in 1995. During the last 20 years he has been working at several private and public institutions related to aquaculture production and research.