

# Studies on development of formulated diets with differentially processed animal protein sources and enzyme complex for marine fish larvae and juveniles

学位名	博士(海洋科学)
学位授与機関	東京海洋大学
学位授与年度	2017
学位授与番号	12614博甲第486号
URL	<a href="http://id.nii.ac.jp/1342/00001586/">http://id.nii.ac.jp/1342/00001586/</a>

# 学位論文の要約

Applied Marine Biosciences

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(海産仔稚魚における動物タンパク質源および酵素混合物の有効性に関する研究)

Public concern has grown about for decrease of Pacific bluefin tuna *Thunnus orientalis* (PBT) resources in wild. Since collected PBT was imported and sold mainly in Japan, this country is responsible for sustainable use of PBT resources. Japan has carried out technical research for establishing PBT aquaculture from 1970s. The first success of complete culture of PBT was established in Kinki University in 2002 and considerable effort has been made to advance stable production technique of PBT. However, survival rate of PBT from hatch to juvenile is less than 2%. One reason of lower survival could be due to limited supply of prey fish such as spangled emperor *Lethrinus nebulosus* and Japanese parrotfish *Oplegnathus fasciatus* and minced sand lance *Ammodytes personatus*. In order to solve this problem, this study aimed to determine optimal protein source for the formulated diet to replace these feed items.

Therefore, three feeding experiments were designed to evaluate availability of three types of fish meal for juvenile PBT, and to evaluate the effect of dietary supplementation of enzyme complex in the formulated diet for juvenile PBT. Furthermore, one feeding experiment was designed to evaluate the effect of non-heated animal protein sources on the diet of juvenile red sea bream (RSB).

In the first experiment, three dietary treatments were employed; Peruvian anchovy meal diet (P), domestic fish meal diet (D), fine grind domestic fish meal diet (F), and prey fish (PF; spangled emperor larvae, control). Survival and growth were compared after day 11 of the feeding trial. The highest survival was recorded in PF group, but not significantly different with the other test diet groups. For the test diet groups, there was no significant difference among all dietary groups. Among the groups, the PF group had significantly greater total length and body weight. In terms of test diet groups, the fish fed P diet based on Peruvian anchovy meal showed significantly higher total length than the other dietary groups.

Second experiment was conducted to evaluate the effect of supplementation of enzyme complex for juvenile PBT. Two test diets were formulated to contain 52.8% of Peruvian fish meal (FM) and FM+0.5% of enzyme complex (EC) with 40% of moisture content in gelatin bound. Commercial diet (CD) and minced sand lance (SL) were used as a control. The survival rate and growth performances of fish in different treatments were compared after 10 days of feeding trial. The highest survival was recorded in SL, followed by CD, FM+EC and FM group, but no significant difference was found. The SL group had significantly highest total length and body weight among treatment groups. For the test diet groups, the PBT juvenile fed FM+EC diet were significantly higher total length and body weight than that of FM diet, but no significant difference was found in CD group.

Third experiment was conducted to evaluate the effect of supplementation of three type enzymes for juvenile PBT. Four test diets were formulated to contain 52.3% of Peruvian fish meal + 0.5% of three enzyme (Enzyme A, Enzyme B and Enzyme C) or 52.0% of enzyme-treated Chilean Horse mackerel meal (ETFM) with 20% of moisture content in gelatin bound. Commercial diet (CD) and minced Sand lance (SL) were used as a control. The highest survival was in the group fed ETFM, followed by CD, SL, Enzyme B, Enzyme A and Enzyme C group, but no significant difference was observed in survival among the groups. ETFM group showed better growth than CD group, but there were no significantly differences in total length and body weight.

In fourth experiment, five test diets were formulated to contain heated squid meal (HS), non-heated

squid meal (NHS), heated krill meal (HK), non-heated krill meal (NHK) and fish meal (FM) as the control. The carcass was sampled at first, third and fifth week after the initial feeding. In terms of growth performance, fish fed the krill meal diet showed better growth than that of squid meal diet during first week of rearing period. However, the squid meal diet group showed better performance than krill meal diet group after third week. The difference in body weight among treatments was greater at fifth week. This suggested that krill meal is effective in body weight of fish during the first week, however, increased in growth enhanced utilization of squid meal in the diet after third week.

In conclusion, a formulated diet is able to replace prey fish larvae without negative impact and improves growth of PBT juvenile. It was demonstrated that addition of external enzyme in diet improved growth performance of PBT and protease and lipase were suggested to be effective in the supplemental enzyme complex. In addition, it was thought that moisture content in gelatin bound formulated diet was 20% in the diet for PBT and the diets with 1.0-2.9 DHA/EPA ratio did not have negative impact on PBT and RSB performance. Dietary free amino acid and water soluble protein seemed to be suitable amino acid and energy source for PBT and RSB during early life stage.