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

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

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論文題目 Title	Studies on development of formulated diets with differentially processed animal protein sources and enzyme complex for marine fish larvae and juveniles (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)		

Production of larvae and juvenile Pacific bluefin tuna (PBT) in commercial hatcheries still depends on the supply of live prey such as rotifer, *Artemia*, yolk-sac larvae and minced fish meat. However, this practice is difficult, expensive, laborious and time consuming to maintain efficiently and often nutritionally incomplete. Even though several efforts have been made to advance and stabilize production technique for this species, survival rate from hatch to juvenile is still less than 2%. One reason of lower survival rate could be due to limited supply of prey fish such as spangled emperor *Lethrinus nebulosus*, Japanese parrotfish *Oplegnathus fasciatus* and minced sand lance *Ammodytes personatus*. It is necessary to replace these feed items by formulated diets for juvenile PBT.

During production, fish meal is heated during manufacturing processes which affect its digestibility and absorption of protein by fish. While raw feed such as prey fish and minced sand lance has high digestibility due to their endogenous enzyme. On the contrary, it is considered that digestibility of a diet was negatively affected by heat processing of protein source of the diet. However, few studies were conducted to examine utility of non-heated protein sources as well as various type of fish meal on growth performance of fish. Further, enzyme treated Chilean fish meal based commercial diet was used for juvenile PBT and suggested positive effect of enzyme for PBT diet. However, there is no study examining dietary supplementation of external enzyme for PBT diet.

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). PBT larvae at the 19th day after hatching with mean total length of 17.5 mm were fed prey fish larvae and the commercial diet during the first four days of the feeding trial and weaned onto each diet to examine the effect of test diets for PBT. 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 feeding trial was stared at the 26th day after hatching when total length of the initial fish was 31.0 mm. Four kinds of dietary treatments were subjected to eight tanks of PBT in duplicate and provided dietary items. 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. PBT juvenile at the 28^{th} day after hatching with mean total length of 38.0 mm were fed prey fish larvae and commercial diet during the first five days of the study, and weaned onto each treatment. Survival and growth were compared after day 17 of the feeding trial. 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. Fifty RSB juveniles (initial mean weight = 3.5g) were fed one of the five diets for 5 weeks, three times daily until satiation. 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.