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Note

Replacement of frozen fish meat based diet with artificial diets in rearing of coral trout *Plectropomus leopardus* (Lacepede, 1802) fingerlings

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ABSTRACT

This study compared the efficacy of four diets in rearing of coral trout *Plectropomus leopardus* during the nursery phase. Details of the experimental diets used in the study are: Diet 1 - prepared with a mixture of golden pompano meat and shrimp meat (2:1, w:w); Diet 2 - prepared with golden pompano meat, shrimp meat, and fish meal (2:1:3, w:w:w); Diet 3 - a commercial pellet with 46% protein (Hairui®, P. R. China) and Diet 4: a commercial pellet with 53% protein (Huacheng®, P. R. China). Fish fed Diet 2 showed the lowest survival rate. The specific growth rate (SGR) of fish fed with Diet 3 and Diet 4 were significantly higher than those fed with Diet 1 and Diet 2 ($p< 0.05$). Food conversion rates (FCR) of fish fed with Diet 3 (1.5 ±0.4) and Diet 4 (2.0 ±0.3) were significantly lower than those fed with Diet 1 (5.5 ± 0.6) and Diet 2 (7.7 ± 1.7, $p< 0.05$). Results from the present study demonstrated that it is feasible to feed coral trout fingerlings with commercial pellets during nursery phase. The fish meat based diet can be replaced with Diet 3 or Diet 4, and among these the most economic diet was Diet 3 with the production cost rate of CN ¥ 0.02 per g biomass.

Keywords**:** Coral trout, FCR, Growth, *Plectropomus leopardus,* RNA/DNA ratio, Survival

Coral trout *Plectropomus leopardus* belonging to the family Serranidae, is distributed in the subtropical and tropical coral areas of Western Pacific, East Africa and the Red Sea (Seno, 2000; Zeller, 2002; McLean *et al.*, 2011). As one of the most important commercial and sport fishery resource, coral trout is very popular in Indo-West Pacific region, and the retail price range from HK$ 365 to 805 per kg (http:/www.fmo.org.hk). Recently, as a consequence of successful artificial breeding and rearing of this species in southern China (Liu *et al.*, 2012; Ma *et al.*, 2013), the market price of coral trout dropped slightly (http://www.fmo.org.hk). However, high production cost and low survival rate during the nursery phase hinder the commercial production of this species.

Several studies have been conducted to compare the efficacy of using trash fish and commercial pellets in rearing marine fish (Usman *et al.*, 2009; Shapawi *et al.*, 2011; Bunlipatanon *et al.*, 2012). However, results are controversial (Bunlipatanon *et al.*, 2012). Coral trout juveniles have strict feeding preferances and our previous studies indicated that coral trout juveniles select only sinking feeds during feeding (unpublished). In China, live *Artemia* and frozen trash fish diet are the primary diets for coral trout during the nursery phase. Fish are normally weaned from live *Artemia* to prepared trash fish meat diet once the body weight reaches 0.9 g. Such feeding regime is based on the farmer's perception that using trash fish diet results in better rearing performance, and that it is hard to wean fingerlings of cultured marine fish collected from the wild with commercial pellets. The present study aimed at comparing the effect of four test diets in improving the growth, survival, food conversion ratio (FCR) and production cost optimisation of coral trout during the nursery phase.

A total of 1,000 hatchery reared coral trout fingerlings (mean wet weight 0.99 ± 0.08 g) were obtained from local hatchery in Lingshui, Hainan Province, P. R. China, and were transported to the Tropical Fisheries Research and Development Center, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Science, Xincun Town. Upon arrival, fish were acclimated in two 1,000 l capacity fiberglass tanks for one week. Acclimation tanks were cleaned daily by siphoning out the bottom sediments and were supplied with seawater filtered through 5 µm filter, maintaining the water exchange rate at 100% day^{-1} per $tank^{-1}$. Two air stones were placed in each tank to maintain the dissolved oxygen at nearly saturation level. During acclimation, fish were fed three times a day with live *Artemia*. Natural photoperiods were used during the acclimation period (14 h light: 10 h dark), and the light intensity was 2,900 - 3,300 lux. The water temperature and salinity were maintained at 29.8°C and 33‰ respectively throughout the experiment.

After one week of acclimation, experimental fish were stocked in twelve 400 l fiberglass tanks at a density of 0.15 fish per litre. All the rearing tanks were supplied with seawater filtered through a 5 µm filter from the top of the tank with a daily water exchange rate of 200% tank volume. Four types of diets were tested in this study *viz*., Diet 1 - mixture of golden pompano meat and shrimp meat (2:1, w:w); Diet 2 - mixture of golden pompano meat, shrimp meat, and fish meal (2:1:3, w:w:w); Diet 3 - commercial grouper pellet with 46% protein (with 3 mm dia, and 3.5 mm length sinking pellet, Hairui®, P. R. China) and Diet 4 - commercial grouper pellet with 53% protein (with 3 mm dia, and 3.5 mm length sinking pellet, Huacheng®, P. R. China). In preparation of Diet 1, sliced fish meat and shrimp meat were blended in a high speed blender, and directly preserved in -20°C freezer for experimental use. Diet 2 was prepared by blending sliced fish meat and shrimp meat in a high speed blender, then mixed with fish meal at 2:1:3 (w:w:w), and preserved at -20°C. According to the information provided on the commercial feed bags, the main raw materials used in Diet 3 and 4 were fishmeal, trash fish, fish oil, squid oil, soybean meal and wheat flour. The proximate analysis of the diets were done in triplicates as per standard procedures (AOAC, 1980), and are summarised in Table 1. Fish were co-fed with live *Artemia* and experimental diets for 5 days. Live *Artemia* was supplied to rearing tanks three times per day at 07:00, 12:00, and 17:00 hrs, and experimental diet was provided at one hour interval from 08:00 to 16:00 hrs.

Table 1. Proximate composition of experimental diets (in % dry mater)

Parameters	Diet 1	Diet 2	Diet 3	Diet 4
Crude protein	16	37.5	46	53
Crude lipid	5.8	12.3	12	12
Crude fibre	0.02	0.5	3	4
Ash	1.4	6.6	16	13
Lysine	3.7	3.1	2.2	2.5
Moisture	69.1	35.6	10	9

After 5 days of co-feeding, weaning was completed and experimental diets became the sole diet in daily feeding. Experimental fish were fed three times per day at 07:00, 12:00, and 17:00 hrs to satiation. In order to minimise the potential loss of Diet 1 and 2 in water, during each feeding, the diets were partially thawed and fed to the fish immediately. The faeces and uneaten diets were removed three times per day by siphoning the bottom. Weight of uneaten feeds/leftover feed from each experimental tank was recorded for FCR determination. The feeding experiment lasted for 30 days.

Experimental fish were randomly collected in triplicates from the rearing tanks on day 1 and day 30, and anaesthetised in 5% AQUI-S (AQUI-S New Zealand Ltd, Lower Hutt, New Zealand). Wet weight was measured using an electronic balance to the nearest 0.001 g. Growth in terms of specific growth rate (SGR) (% day^{-1}), was calculated as: SGR = $100(\ln W_f - \ln W_i)/\Delta t$, where W_f and W_i were the final and initial larval fish wet weight, respectively, and Δt was the time lapse between sampling intervals. On termination of the experiment, the number of fish in the experimental tanks were ascertained to estimate the final survival rate. Food conversion ratio (FCR) was calculated using the formula: FCR = (Feed fed – Uneaten feed from the tank) / Weight gain. The production cost rate (PCR, in CN ¥ per g fish) was calculated as: PCR = Cost of the diets per fish / Weight gain per fish. The unit price of experimental diets were CN ¥ 0.03 per g for Diet 1, CN ¥ 0.032 per g for Diet 2, CN ¥ 0.015 per g for Diet 3, and CN ¥ 0.09 per g for Diet 4.

Four fish from each rearing tank were collected for the analysis of RNA/DNA ratio, at the end of the experiment. Fish were anaesthetised in 5% AQUI-S and were pre-washed with distilled water and then snap-frozen immediately in liquid nitrogen. Frozen samples were dissected in an ice tray, and muscle tissue samples were collected from the fish. The RNA/DNA ratio was determined following the method described by Schneider (1957) and Zehra and Khan (2013). Each pooled sample was weighed to the nearest 0.001g in an electronic balance and placed in a test tube in an ice slurry bath. The tissue samples were then homogenised in 5% trichloroacetic acid (TCA) at 90°C and centrifuged at 5,000 g for 20 min. For RNA determination, 2 ml of distilled water and 3 ml of orcinol reagent were added to 1 ml of supernatant. The reaction mixture was kept in boiling water for 20 min. The greenish blue colour thus developed was read at 660 nm. For DNA determination, in 1 ml of supernatant, 1ml of distilled water and 4 ml of freshly prepared diphenylamine reagent were added. The reaction mixture was kept in a boiling water bath for 10 min. The blue colour developed was measured at 600 nm. Standard curves for RNA and DNA were drawn using different concentrations of yeast RNA and calf thymus DNA (Sigma-Aldrich, USA), respectively. The dataset was expressed as mean ± SD and tested using one way ANOVA (PASW Statistics 18.0; Chicago, IL, USA). Significantly different means were then elucidated using the Tukey's test.

The growth of coral trout juveniles in terms of SGR was significantly affected by the type of diets ($p < 0.05$, Table 2). The SGRs of fish fed with Diet 3 and Diet 4 were significantly higher than those fed with Diet 1 and

Table 2. Specific growth rate (SGR), survival, RNA/DNA ratio, food conversion ratio (FCR), and production cost rate (PCR) of *Plectropomus leopardus* juveniles fed with four different diets for 30 days

	Diet 1	Diet 2	Diet 3	Diet 4
SGR (%/day)	4.96 ± 0.22a	5.79 ± 0.32a	7.00 ± 0.20b	7.16 ± 0.31b
Survival (%)	81.33 ± 15.68a,b	60.24 ± 8.47a	95.48 ± 4.52b	74.07 ±14.58a,b
RNA/DNA	0.78 ± 0.32	0.71 ± 0.28	0.50 ± 0.23	0.77 ± 0.32
FCR	5.55 ± 0.68b	7.67 ± 1.71b	1.53 ± 0.15a	2.04 ± 0.56a
PCR (CN¥ per g fish)	0.26 ± 0.17b	0.25 ± 0.05b	0.02 ± 0.00a	0.18 ± 0.05b

Diet 2 ($p< 0.05$, Table 2), and the SGR of fish was not significantly different between Diet 1 and Diet 2, or between Diet 3 and Diet 4. The final survival rate of fish fed with Diet 3 was significantly higher than fish fed with Diet 2 ($p< 0.05$, Table 2). Upon completing the 30 days feeding trial, it was found that the RNA/DNA ratios of coral trout juveniles were not significantly affected by the diets ($p< 0.05$, Table 2). The RNA/DNA ratios of fish varied from 0.50 ± 0.23 to 0.77 ± 0.32.

The FCR of fish was significantly affected by the feeds ($p < 0.05$, Table 2). FCR of fish fed with Diet 3 and Diet 4 was significantly lower than those fed with Diet 1 and Diet 2 ($p < 0.05$, Table 2). No significant differences were found between fish fed with Diet 1 and Diet 2 or between Diet 3 and Diet 4 ($p> 0.05$, Table 2). In this study, the lowest production cost rate *i.e.*, CN ¥ 0.02 to produce 1 g biomass was obtained in the group fed with Diet 3. The production cost of fish were not significantly different when fish were fed with Diets 1, 2, and 4 ($p>0.05$, Table 2), and varied from CN¥0.18 per g biomass to CN ¥ 0.26 per g biomass.

Although lot of effort have been made to develop appropriate type of diets to culture marine fish (Tacon *et al.*, 1991a; b; Bombeo-Tuburan *et al.*, 2001;), till date, the conclusions are not satisfactory. Switching to formulated feeds has been considered as the essential pathway to sustainable marine fish aquaculture (Hasan, 2012). However, achieving the transition is difficult owing to the following reasons: (i) restricted by the structure of supply chain in formulated feeds, (ii) lack of an operational understanding of farmers' perceptions of the comparative benefits of the use of low value fish and formulated feeds and a scientific assessment of their farming practices and livelihood strategies, (iii) lack of organised scientific information and technical assistance to persuade farmers and to serve as guidelines for governments to formulate policy (Hasan, 2012). In this study, we have successfully compared the rearing performance and production cost rate of coral trout fed with four commonly used diets in nursery phase.

After 5 days co-feeding with live *Artemia*, experimental fish can be successfully weaned to the target dietary types, and no mass mortalities were observed during the weaning period. The growth and survival rate of coral trout were significantly affected by the dietary type ($p<0.05$). A better growth performance was obtained when fish were fed with commercial pellets (Diets 3 and 4). The survival rate of fish was not significantly different when fed with Diets 1, 3, and 4. Although the survival of fish fed with Diet 1 was similar to Diets 3 and 4, we suggest avoiding it as there is a risk of transmission of parasites from the feed to the cultured fish, resulting in increased potential mortality due to the disease outbreak (Ruckert *et al.*, 2009).

The use of RNA/DNA ratios to understand fish growth has been demonstrated in a variety of fish species (Gwak *et al.*, 2003; Hook *et al.*, 2008; Giotti *et al.*, 2010). It has been suggested that RNA/DNA ratios in fish larvae and juveniles are closely related to food availability and feeding condition, and therefore can be used to assess the nutritional condition of fish (Chicharo, 1998; Giotti *et al.*, 2010; Tong *et al.*, 2010; Diaz *et al.*, 2011). Furthermore, the RNA: DNA ratio can be used as an index of the growth potential of cultured fish (Bulow, 1987; Tanaka *et al.*, 2007). In the present study, the RNA/DNA ratio of fish was not affected by the dietary type suggesting adaptation of fish to these four types of feeds. However, the growth rate of fish with different dietary types disagree with their RNA/DNA ratio which remain unclear.

Studies indicate that the FCR is poor when low - value trash fish is used as the primary food source in average grouper farming practices, and the FCR of such feeds range from 7:1 to 15:1 (De Silva and Turchini, 2009). According to Hasan (2012), the FCR of fish fed with pellets is about 2.5 and is around 12-13 when the fish are fed with low value trash fish. Similar results were also observed in the present study. The FCR of coral trout fed with diets prepared using golden pompano meat and shrimp meat (Diet 1 and 2) was significantly higher ($p<0.05$) than with commercial pellets (Diets 3 and 4). FCR of fish fed with trash fish prepared diet (Diets 1 and 2) was between 5.6 and 7.7, which was significantly higher than those of fish fed with pellets (Diets 3 and 4, with FCR between 1.5 and 2.0). This result clearly indicates the advantage of using pellets in rearing of coral trout during the nursery phase.

In the present study, the experimental Diets 1 and 2 were prepared strictly by the formula followed in the local fish farm, using golden pompano meat and shrimp meat, hence the unit price of Diets 1 and 2 were higher than that of pure trash fish diet. Upon completing 30 days feeding trial, the production cost rate of fish fed with Diet 1 (CN ¥ 0.26 per g biomass), Diet 2 (CN ¥ 0.25 per g biomass) and Diet 4 (CN ¥ 0.18 per g biomass) were significantly higher ($p<0.05$) than that of Diet 3 (CN ¥ 0.02 per g biomass). Although the FCR of fish fed with Diet 3 and 4 were similar, the unit price of Diet 4 with 53% protein content was significantly higher ($p<0.05$) than Diet 3 with 46% protein content; which possibly led to a high production cost rate for Diet 4.

Based on the results obtained in the present study, it is concluded that trash fish prepared diet can be effectively replaced by commercial pellets, and commercial pellets with 46% protein content are recommended in rearing of juvenile coral trout during nursery phase.

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