nile gold spot grouper and compared with feeding either a commercial pellet diet or fresh fishery bycatch. In both studies, fish fed the project formulation diet survived and grew as well as those fed the fresh bycatch. In the laboratory study, fish fed the commercial pellet diet grew significantly slower and converted feed less efficiently than those fed either the project diet or fresh bycatch. The analysis of the commercial pellet diet showed a sub-optimal specification. When the commercial mill adjusted the formulation to meet these specifications, fish fed that diet in the field study performed as well as those fed either the project diet or fresh bycatch.

Conclusions

The research carried out in the project has conclusively shown that juvenile groupers will readily accept pelleted dry diets. Diets formulated to meet the fish's requirements for digestible nutrients, and not containing excessive amounts of plant protein meals, will enable juvenile groupers to grow as well, if not better, than those fed fresh fishery bycatch. Further research is needed in the areas of essential fatty acid requirements of tiger groupers (*E. fuscoguttatus*) and to examine whether the nutritional requirements of groupers above 200-300 g are different to juveniles of 10-100 g size. Another area of potential research is to develop nursery feed formulations and to develop management practices for successful weaning of fry from live/fresh feeds to a pelleted dry feed.

Feed development and application for juvenile grouper

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Groupers are potentially important aquaculture species since they have a high economic value. Groupers, predominantly *Epinephelus spp*, have been cultured throughout Asia for many years with commercial production based on captured wild seed and the fish reared on trash fish. Recently, hatchery technology has been developed for seed production of some grouper species and this has stimulated interest in grow-out farming. However, the continued use of trash fish as a feed source for groupers should be discouraged because of the risk of disease transfer and the environmental problems associated with its use.

Feed is often the single largest cost item in fish culture. Although trash fish is presently the first choice of farmers for on-growing groupers, its availability can be limited and varies with season. Information on the nutrient requirements of groupers is still very limited and it is imperative that this is addressed if cost-effective and high performing artificial feeds are to be developed to replace the feeding of trash fish. Based on the limited available information on the nutrient requirements of groupers, some feed companies have produced feeds for nursery and grow-out. However, these have not been well accepted by grouper farmers because they are thought to be expensive and the fish do not accept it as well as trash fish. In order to develop a better artificial feed for juvenile groupers, a series of experiments have been conducted at Gondol to increase our knowledge about the dietary requirements of several grouper species.

Nutrient requirements of juvenile groupers

Dietary protein and lipid requirements

Tiger grouper (*Epinephelus fuscogutatus*) and humpback or mouse grouper (*Cromileptes altivelis*) are carnivorous fish and thus naturally have a high requirement for dietary protein. Grow-out studies have examined the optimum dietary protein and lipid specifications for juvenile tiger and humpback groupers. A significant interaction between dietary protein and lipid has been observed for growth rate. Although fish productivity generally increases with increasing dietary protein, feeding with 9% lipid feed was found to be better than feeding either the 6 or 12% lipid feeds. Food conversion ratio (FCR) improved and more dietary lipid was retained as dietary protein increased; retention of dietary lipid also increased with increasing lipid content of the diet. There was a slight difference between tiger and humpback groupers in the optimal dietary protein specification: 47% for juvenile tiger grouper and 54% for humpback grouper. In another series of experiments, five levels (0.0, 3.0, 6.0, 9.0, and 12%) of dietary lipid were fed to juvenile tiger and humpback grouper. This confirmed the earlier results with the optimal dietary lipid found to be 12 and 9%, respectively for juvenile tiger and humpback grouper. Thus for juvenile tiger and humpback groupers, pelleted diets of about 90% dry matter (DM) should contain around 50% protein, 9-12% lipid, about 4.5 kcal gross energy/kg (18.8 MJ/kg) and a protein to energy ratio of 120 mg/kcal (27 g/MJ).

Essential fatty acid requirements

Omega 3 or n-3 highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential dietary fatty acids for marine fish. Requirement for n-3 HUFA varies with the species and size of fish. Experiments have examined the n-3 HUFA requirement of juvenile humpback and tiger groupers. Juveniles of average starting body weight of 5 ± 0.7 g were reared for 9 weeks in 100 L tanks and supplied with flow through seawater. Fish were fed one of six feeds that provided n-3 HUFA levels of 0, 0.5, 1.0, 1.5, 2.0 or 2.5%. Based on growth rate and FCR, the optimal dietary n-3 HUFA specification was found to be 1.5 - 2.0% for humpback grouper and 1.75 –2.8% for tiger grouper.

Vitamin requirements

Similar to all other carnivorous marine fish, groupers require a dietary source of vitamin C (ascorbic acid). A lack of vitamin C in very young fish will result in skeletal deformities such as lordosis and scoliosis (curvature of the skeleton) and deformity or absence of the gill opercula; in older fish a deficiency is exhibited as slow growth, weak sickly fish, anemia and death in severe cases. Vitamin C is very important for maintaining a strong immune system and any deficiency will increase the fish's susceptibility to stress and disease. Pure ascorbic acid is very easily destroyed by mild heating and exposure to light. To overcome the low stability of ascorbic acid, other more stable types of vitamin C have been developed. Some products are simply ascorbic acid coated by glycerine or some other film which helps to reduce exposure of the ascorbic acid to light and so slow its destruction. However, these coated ascorbic acid products are still not very stable and are mostly destroyed during any type of hot pelleting process. Ascorbyl phosphate is a much more stable form of vitamin C, which has been shown in other fish species to be effective in meeting the fishes nutritional needs. To evaluate its effectiveness for groupers, juvenile humpback groupers of about 14 g starting weight were fed feeds containing 0, 15, 30, 60, 120 or 250 mg/kg of ascorbyl phosphate magnesium (APM) for 126 days. Based on the results of this experiment, it is recommended that pelleted grouper feeds should contain not less than 30 mg APM/kg for maximum fish growth and to ensure that the fish's immune system is fully functional.

Another experiment has been conducted to determine the requirement of vitamin B6 (pyridoxine). This is another important and essential vitamin for fish and in severe deficiency neurological disorders such as erratic and spiral swimming, shock reaction to stress or handling and death are seen. In a less severe deficiency, the signs are fairly non-specific such as poor appetite and slow growth rate of the fish. Fortunately, pyridoxine hydrochloride, which is the commonly available form of pyridoxine, is very stable to heat and light. To determine the pyridoxine requirement of groupers, six levels of pyridoxine (0, 20, 40, 60, 80 or 160 mg/kg) were fed to juvenile humpback groupers for 98 days. Using the increase in blood haemoglobin as an index of pyridoxine adequacy of the feed, the optimal specification for maximum blood haemoglobin was 60 mg/kg. This is much higher than the 15-20 mg/kg dietary specification recommended for many other marine fish. Since pyridoxine HCl is quite soluble and groupers are fairly slow feeders, this higher specification may be due to leaching of pyridoxine from the feed. In any event, it is recommended that grouper feeds have a dietary pyridoxine HCl specification of 40-60 mg/kg to maintain the healthy condition of fish.

Table 1. Formulation and macro nutrient composition of the Gondol practical feed formulation for juvenile groupers

Ingredient	Formulation (%)	Nutrient	Composition (% of diet)
Fishmeal (65% CP)	55	Crude protein	46-50
Soybean meal	10	Total lipid	9-13
Squid liver meal	14.5	Fiber	6-7
Mysid shrimp meal	6	Ash	18-25
Squid oil	6		
Trace mineral mix	2.5		
Vitamin mix	2.0		
CMC binder	4.0		
Total	100		

Table 2. Result of a 4-month feed comparison trial with humpback grouper in net cages

Productivity traits	Test diets		
	Gondol feed	Commercial feed	Trash Fish
Initial weight (g)	36.0	36.0	36.0
Final weight (g)	147.6	132.8	133.4
Survival rate (%)	98.7	98.0	95.1
FCR	1.39	1.54	5.82
Haematocrit (%)	37.3	38.2	24.2

Practical feed development for juvenile groupers

Fishmeal replacement

Fish meal is the main source of protein in conventional pelleted feeds for most fish species. Because capture fisheries production has not increased since the early 1990's and production of fishmeal has similarly not increased but aquaculture's demand has skyrocketed, it is not surprising that the price of fishmeal continues to increase at rates above inflation. In an attempt to offset the spiraling cost of fishmeal as an ingredient in pelleted fish feeds, there has been a lot of research worldwide to find cheaper protein alternatives. At Gondol, we have examined the extent to which soybean meal can be used to replace

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fishmeal in grouper feeds. Unfortunately, using soybean meal in amounts exceeding 10% of the diet as a protein replacement for fishmeal caused growth rate and food conversion of the fish to worsen.

Practical feed formulation for juvenile groupers

A suitable practical feed formulation for juvenile groupers has been developed at Gondol (Table 1).

In a 4-month trial with humpback grouper held in a floating net cage, the effectiveness of the Gondol feed formulation has been compared against a commercial pelleted grouper feed and trash fish.Table 2 shows the results of this comparison. Growth rate and FCR were best for grouper fed the Gondol feed. In terms of growth rate and FCR, the commercial feed was as good as the vitamin-supplemented trash fish. Interestingly, the haematocrit value (proportion of red cells in the blood – an indirect measurement of haemoglobin content) of the grouper fed trash fish was quite low, suggesting either a vitamin deficiency (even though a vitamin supplement was mixed with the trash fish) or some other toxic or infectious agent present in the grouper.

Future research

Gondol will continue research to increase our knowledge about the nutrient requirements of groupers with tiger grouper being the main species to be used for these investigations. A priority area is to develop successful nursery feeds in order to quickly and easily wean fry from live feed (or trash fish) to pelleted dry feeds. This work will be coordinated with other grouper feeds research that will be carried out in the ACIAR Marine Finfish Technology Improvement project at Maros (Indonesia).

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